

Analyses of morphology and mitochondrial DNA reveal a deep split within

Parnassius charltonius GRAY, [1853]

(Lepidoptera, Papilionidae)

by

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Abstract: The article presents the synthesis of mtDNA, morphological and geographical data on different groups of taxa united under the name *Parnassius charltonius* GRAY, [1853]. *Parnassius romanovi* GRUM-GRSHIMAILO, 1885 **stat. nov.** constitutes a bona species distributed in Alai, Ghissar, West Pamirs/Badakhshan and the SW edges of Tian-Shan. It is characterized by a reduced uncus (which is shorter than the tegumen) and a reduced third submarginal spot on the HW underside. The status of the taxa *deckerti* VERITY, 1907 and *voighti* BANG-HAAS, 1927 is questionable and needs further clarification. Especially the latter taxon could constitute a separate species. Comparative analysis of mtDNA and morphological data makes it possible to interpret the action of evolutionary factors in the history of the taxa.

Introduction: The butterfly family Papilionidae has recently been the object of numerous molecular and phylogenetic studies, many of which included representatives of *Parnassius* LATREILLE, 1804 (OMOTO et al., 2004; KATOH et al., 2005; NAZARI et al., 2007; GRATTON et al., 2008; MICHEL et al., 2008; OMOTO et al., 2008; TODISCO et al., 2010, 2012; CONDAMINE et al., 2012). During the same period, field studies resulted in the discovery of a number of new taxa in the subgenus *Kailasius* MOORE, 1902, which includes some of the most spectacular members of *Parnassius* LATR. One new species (*P. davydovi* CHURKIN, 2006) and at least six new subspecies of *P. charltonius* GRAY, [1853] were found and described recently, the distribution area of the latter being significantly enlarged as a result. During these studies, it was found that the structure of the σ genitalia of *P. charltonius* GRAY differs significantly from that of other representatives of *Kailasius* MOORE (CHURKIN, 2006) and, moreover, that different *charltonius* taxa have remarkably different genitalia (CHURKIN & PLETNEV, 2012).

The necessity to confirm and extend these findings prompted us to undertake an in-depth comparative study of taxa united under the name „*Parnassius charltonius* GRAY“ and to this end, we chose to associate morphological analyses with the sequencing of DNA. Such combined analyses have been published for various Vertebrate complexes, but are not yet commonplace for insects, including butterflies (for Parnassiinae, the study of the *Zerynthia polyxena* complex by ZINETTI et al., 2013, constitutes a recent exception).

The primary goal of this work was to clarify the status of the northern populations (united under the oldest available name *romanovi* GRUM-GRSHIMAILO, 1885), but our results, which reveal a deep, ancient split within *P. charltonius* GRAY, also raise questions about the microevolution, adaptive radiation and multidimensional analysis of the species involved. At the same time, it should be noted that ascertaining the status of individual subspecies is beyond the limits of the present article, if only since this will require systematic work based on lectotype designations that are necessary to restrict the type localities. Unfortunately, that remains to be done for many *Parnassius* taxa, including *P. charltonius* GRAY (only in the *romanovi* group the taxonomic work was finished recently by the senior author of the present paper).

Abbreviations: FW - fore wing, HW - hind wing, TL - type locality, mtDNA - mitochondrial DNA

I. Morphological studies

1. Groups of taxa and distribution

The distribution area of *P. charltonius* GRAY (fig. F1) extends from Western Tibet (close to its frontiers) and Western Nepal to Bamyan in Afghanistan; its northern limits are the Alai and Turkestan ranges and the south-western part of Inner Tian-Shan. It is not known from the main territory of Tian-Shan, West Ghissar or the western edges of Afghanistan. According to different authors, the subspecific structure of *P. charltonius* GRAY is formed of three or four groups (plate 1-2). KREUZBERG (1985) confirmed the existence of four groups (“*deckerti*”, “*charltonius*”, “*romanovi*” and “*voighti*”), but distinctive characters were not listed. ROSE & WEISS (2011) used three groups only, uniting the *romanovi* and *voighti* groups, but again without a detailed analysis of distinctive features.

The *romanovi*-group occupies the northern part of the species range, i.e. Ghissar, Alai, Transalai (including the upper stream of the Chinese Kyzyl-Su river.), Darwas, West Pamirs and the SE part of Tian-Shan. In total, 11 subspecies are known, mainly with deep hiatuses in colouration; the detailed situation will be discussed separately.

For the sake of comparative analysis of group distinctions, it is important to note the clinal structure of the characters found among populations of *vaporosus* AVINOV, 1913 - *kabiri* EISNER & NAUMANN, 1980 (CHURKIN & PLETNEV, 2014). While butterflies from the Vanch range (West Pamirs) are the most variable ones within this complex, that population has no possibility to contact individuals from the *voighti* and *deckerti* groups. In contrast, butterflies from the Dzhilandy or Ishkashimsky ranges (which lie closest to the distribution areas of the *voighti* and *deckerti* groups) exhibit less varia-

bility and lack forms with characters unexpected for the *romanovi* group.

The *voigti*-group is restricted to Afghanistan, from Bamian to Nuristan and from the Kabul area (Paghman Mts.) to the Khodja-Muhammad range, i.e. it extends on both sides of the Hindukush. At least two subspecies exist in the literature (*wernickei* KOTZSCH, 1936 and *voigti* BANG-HAAS, 1927), but three additional taxa have been described and it cannot be excluded that some of them represent good subspecies as well.

It is important to note that sometimes the type locality of *wernickei* KOTZSCH is given as “Mujan Pass, Nuristan” (ROSE & WEISS, 2011), while this pass is not located in Nuristan (neither according to official administrative divisions, nor zoogeographically), and the true locality is Nuksan Pass, Hindukush, prov. Badakhshan, not far away from Munjan. The distance between the Nuksan Pass and Malvodz (in the Ishkashimsky range), which is the southernmost locality of both *vaporosus* Av., and the entire *romanovi* group, is less than 60 km. On the other hand, Birmoglasht (Pakistan), a known locality of *ducalis* BOULLET & LE CERF, 1912, which is located 65 km from Nuksan Pass, is separated from the latter by serious natural barriers. As for Baroghil Pass, the TL of the taxon *robertjan* EISNER, 1959 (usually treated as a synonym of *ducalis* BOULLET & LE CERF of the *deckerti* group), which is located 160 km away along the Pjandj (Pjandzh)/Pamir river in the eastern direction, it can be reached practically without encountering any significant barriers.

Contrary to a common opinion, taxon *nuristanus* EISNER & NAUMANN, 1980, which is distributed in the southern direction from the Hindukush range, cannot be a synonym of *wernickei* KOTZSCH, even on zoogeographical grounds. Indeed, the description and available photos of the type series of *nuristanus* EISNER & NAUMANN (HAEUSER et al., www.globis.insects-online.de) reveal its similarity with *voigti* BANG-HAAS, which is more logical (its actual status cannot be finalized without lectotype designations and new material). The straight-line distance between the TL *nuristanus* EISNER & NAUMANN and Birmoglasht is also less than 70 km, although again with serious barriers.

To summarize, while the distribution areas of the three groups of subspecies almost meet at the edges of the SW Pamirs and in the basin of the Pamir river, hybrid populations, or even true hybrid specimens are absolutely unknown.

The nominate taxon occupies the most south-eastern part of the *P. charltonius* distribution area, located between Mounts Kailas, Nanda Devi and Gurla Mandhata. It seems that this area is separated from the range of similar *bryki* HAUDE, 1912, which extends from Nelang (Nilang) Pass to Spiti (north-west from Poo) and Chumurti. This needs confirmation, but we have not yet found any butterflies collected somewhere between Nelang and Nanda Devi (or Kailas) and Chumurti; any information concerning this point would be very important.

The complex *serenissimus* BRYK, 1932- *sakai* EISNER, 1978 - *eisnerianus* BRYK, 1931, which inhabits the southern border of the species distribution, is usually included in the *charltonius* group (without arguments), but we cannot agree with this decision: as discussed in the present paper, several of the characters that we found useful to define major entities show that these subspecies instead should be included in the *deckerti* group. Some differences between the two complexes (*eisnerianus* vs *deckerti*) will be discussed when necessary.

P. ch. flaugeri EISNER, 1978 shares many characters with the *eisnerianus*-complex but is usually included in the *deckerti* group (in addition, it occupies the southern part of the Pakistan distribution area, topographically connected with the ranges of other taxa of the *eisnerianus*-complex). *Parnassius c. ella* BRYK, 1932 was treated as a synonym of *deckerti* VERITY, 1907 (WEISS, 1991), but later as a synonym of *serenissimus* BRYK (ROSE & WEISS, 2011). The type material from Deosai (available at the site www.globis.insects-online.de) belongs to *deckerti* VRTY. without any doubts, yet we did not study types from Skoro-La - such a situation underlines again that the detailed taxonomic study of Indian and Pakistani material remains impossible without lectotype designations.

To summarize, all the territory located in the western direction from the *charltonius*-*bryki* area is populated by the *deckerti* group, which can be divided into a *deckerti*-complex (with reduced HW underside blackish suffusion and enlarged blackish submarginal suffusion) and an *eisnerianus*-complex (with well developed HW underside pattern but reduced blackish submarginal suffusion at the HW upperside). The latter consists of several subspecies and some additional taxa of unclear status.

The *deckerti*-complex itself consists of a series of poorly differentiated subspecies, whose actual subspecific characters and hiatuses have never been studied; in such a situation, any opinion about the status of described component taxa remains just subjective. It is quite logical to assume that we are dealing with the outcome of a process of rapid expansion by an ancestral population which lived originally in a relatively small area. In contrast with other subspecies, the ancestor of the *deckerti*-complex was well adapted to the coldest mountain deserts, which allowed it to cover a giant territory after the end of the glaciations. At present we observe a mix of moderately homogenous populations settled in the territory now freed from the ice shield. Of course, the actual situation must be more complicated (if only since there were several glacial periods), but basically the picture must be correct. In contrast, the southern taxa (as well as those from Kyrgyzstan and Afghanistan) were able to follow their ecological niches and change their optimal altitudes, “moving” up and down along the river valleys or long macroslopes, so that the pressure from climate and natural selection was not so strong.

The East Pamirian Plateau (Mynkhadzhir Mts.) in Tajikistan, which constitutes the western limits of the *deckerti*-area, is populated by *anjuta* J. J. SHCHETKIN & KAABAK, 1985. Another taxon - *mistericus* KAABAK, SOTSHIVKO & TITOV, 1996, described from the Sarykolsky range (Dunkeldyl lake) - is usually treated as a synonym of the previous one and

both of them are often regarded as synonyms of *ducalis* BOULLET & LE CERF. We prefer to keep the first name, as applied to fully isolated Pamirian populations. We make wide use of the specimens from Pamir in our work, as this is a place where representatives of the *deckerti* group occur in close proximity to the areas of other groups (CHURKIN & PLETNEV, 2012).

The status of the two taxa known from East Pamirs is not as simple as might be supposed. Butterflies from Dunkel-dyk Lake have a more pointed apex, a narrowed whitish submarginal band and some additional small distinctions - it has not yet been clarified whether these characters are of ecological origin or rather constitute markers of an isolated population. We cannot exclude that the two phenotypes represent the outcome of two different waves of emigration of *deckerti*-like butterflies to the Plateau, something which constitutes an interesting object of study for the future. Additionally, it is worth mentioning that the paper about the characters of *anjuta* SHCHETKIN & KAABAK published by KAABAK (KAABAK & LESIN, 1994) included several serious technical misprints (L. KAABAK, pers. comm.).

The taxon *mazhaensis* HUANG, 1994 was described basing on 1 ♂ only, the description and figure show that this specimen has all characters of *deckerti* VERITY. The final status of this taxon cannot be proved without new material, but the type locality is quite important. It is located on the northern slopes of Karakorum (not far away from Peak K2). This means that the territory between East Pamirs and Raskem must also be included into the area of the *deckerti* group. This fact is remarkable, because the main chain of Karakorum (more than 6000 m a.s.l.) represented the “absolute” barrier for the species during the glacial periods. Thus, if any *charltonius*-race lived there before the glacial era, it must have been very different from all other known taxa (as is the case for *aenigma* DUBATOLOV & MILKO, 2003 distributed in Kashgaria). Thus the high similarity between the Chinese taxon and other *deckerti* taxa confirms our hypothesis regarding the recent expansion of the *deckerti* complex.

2. External distinctions of the groups

All people who studied *P. charltonius* GRAY proposed that the different complexes of subspecies may be characterized by the degree of development/reduction of the FW postdiscal band, HW submarginal spots, size of red spots, etc. However, the differences were never formalized; moreover, a thorough comparison demonstrates that some characters, typical for one group of subspecies, can be found among representatives of other groups. It means that we are dealing with a complicated pattern of similarity and relationship which makes problems of the connection that cannot be solved in a simple way.

Our own detailed comparisons lead to the following system of differences:

1. In general, the *romanovi* group is characterized by reduced HW submarginal spots, which are very small in *romanovi* GR.-GR. or *vaporosus* Av., but larger and extended (oblong) in *aenigma* DUBATOLOV & MILKO or *sochivkoi* CHURKIN, 2009. Usually, these spots are exactly oblong-shaped, being narrowed at the external (marginal) side, whereas in all other groups, these same spots are moderately square-shaped, with not-narrowed, but wide external sides, i.e. the spots are mainly wider than longer. Specimens with an unclear or unexpected form of submarginal spots are very rare in all groups, only *voigti*-representatives exhibit a pattern of great variability (plate 3).

In reality, this character merely reflects the principal distinguishing feature which separates the *romanovi* group from all other *charltonius* taxa, namely, the differential development of submarginal spots on the underside and upperside of the HW.

In the *romanovi* group, the HW submarginal spots are reduced in size on the upperside, whereas in all other groups, these spots are practically equal in terms of size and density of the blackish scales on the upperside and underside of the HW. This is especially true of the central (third) M-spot, located between veins M2 and M3, which provides the most taxonomically valuable character: it looks widely separated from the marginal blackish line because the external part of the spot is reduced, the blackish scales are replaced by the whitish semitransparent scales of the ground colour (col. pl. 3-4). By contrast, in all taxa that do not belong to the *romanovi*-complex, this third spot has the same size on the upperside and underside of the HW and the additional narrow whitish external strip is absent. It is worth adding, that in the *charltonius* group, this third spot often is moved away from the marginal line (but not reduced in size), whereas in all western and northern *deckerti* taxa, the third spot is not moved, but practically touches the marginal line.

The distinguishing feature described above is practically absolute and the exceptions are extremely rare (col. pl. 4: A, B shows the specimens of *kabiri* EISNER & NAUMANN from Vanch and *deckerti* VRTY. from Lamayuru with the size moderately abnormal for their groups). Some *P. ch. voigti* BANG-HAAS specimens have the third spot slightly reduced with some more or less obvious whitish scales on the lateral sides of the spot. Such a combination can rarely be found in eastern *deckerti* populations (but we did not observe it among the butterflies from the East Pamirs, at the northern frontier of *deckerti* area). In addition, it can be found among representatives of *kabiri* EISNER & NAUMANN from Vanch (but has not been recorded in other *kabiri* populations). It should be added, that representatives of the *deckerti* and *charltonius* group have submarginal spots on the HW underside contrasting, i.e. ‘expressed’ - whereas in *romanovi*- and *voigti*-representatives, this pattern is obviously less contrasting, not well expressed (and, as noted above, in the *romanovi* group, the M-spot is seriously reduced, while in the *voigti* group this spot has practically the same size on both sides of the HW).

The reduction of the third submarginal spot confirms a well-known fact - that the two “upper” spots (i.e. those located between the M1-M2 and M2-M3 veins) and the two spots placed below (between the M3-Cu1 and Cu1-Cu2 veins)

are genetically independent. The evolution of subgenus *Koramius* MOORE, 1902 is characterized by the reduction of the two upper spots, and we found the beginning of the same process among members of the closely related subgenus *Kailasius* MOORE, which looks quite logical.

2. The reduced FW postdiscal blackish band represents another important character: all *romanovi* ♂♂ have only a few blackish scales or a slight and sparse suffusion between veins M3 and Cu2 in the central part of the FW, while the ♂♂ of other groups have a big united black spot (as a rule, with a triangular shape). All *deckerti* ♂♂ with partly reduced spots represent very rare variants; moreover, such ♂♂ are more frequent on the eastern side of the distribution area and are absolutely not known from the East Pamirs. This character is not distinctive in ♀♀, which tend to have a reduced black pattern in all known taxa - however, ♀♀ with a well-developed central spot are typical of the northern and western parts of the *deckerti* range (inhabited by the *deckerti*-complex itself), while in the southern subspecies of this group, light ♀♀ with a reduced pattern are not so rare.

The ♀♀ of the *voighti* group always have a reduced central postdiscal spot, which is composed of two zigzag lines.

3. The development of the HW anal red spot (from Cu2-2A to the anal margin of the wing) sets the *voighti* group apart: *voighti* ♂♂ always lack this spot. However, *bamianicus* HEINKELE, 2003 described recently based on 1 ♂ only, has this anal mark well developed. Moreover, 1 ♂ syntype of *wernickei* KOTZSCH exhibits an anal spot with some reddish scales. The red anal mark is sometimes absent in nominate *vaporosus* Av. and *kabiri* EISNER & NAUMANN, but this happens only in smallest specimens.

4. The development of the white pupils inside of the HW red spots is variable, but all *deckerti* and *charltonius* representatives have one white pupil within the big HW red spot, while a white pupil is rarely observed inside of the costal eye for the nominate subspecies. At the opposite, some taxa belonging to the *romanovi* group have two developed white pupils in the big spot and a white pupil in the costal “eye” as well.

In this matter, the underside again demonstrates more valuable characters: all *romanovi* group taxa possess a well developed whitish pupil in the small costal spot and two pupils inside of the big “eye”; these pupils tend to be enlarged, to the point that they often cover nearly all of the space within the “eyes”; exceptions are practically absent. In general, only in a few representatives of *kabiri* EISNER & NAUMANN and *vaporosus* Av. are these pupils not so expressed.

Representatives of the *deckerti* group always show more or less reduced whitish pupils on the HW underside, with the big spot possessing only one pupil which is never enlarged, while the costal “eye” usually has no whitish scales at all.

Nominate *charltonius*-specimens often have one small but bright whitish pupil inside of their costal spot, another one inside of the big “eye” and yet one more inside of the anal reddish mark - the latter feature is rare in other taxa, but can be found among some *deckerti* or *romanovi* ♀♀.

Specimens of the *voighti* group usually have one pupil in the costal spot and one in the big one, but *wernickei* KOTZSCH is characterized by two large pupils in the big spot, especially in ♀♀.

The analysis of other details of colouration suggests that individual characters have different patterns of variability in the groups being studied. Usually, this means that one is dealing with different species.

Representatives of the *voighti* group exhibit great variability in the development of the HW upperside submarginal spots in general - sometimes they are enlarged, sometimes reduced, and in the latter case, different types of reduction are observed: the small spots may be extended and narrowed at their distal extremity, or sometimes these spots are very short, but widened distally (col. pl. 3).

Some subspecies demonstrate complete stability of the “additional” black line (CHURKIN & PLETNEV, 2012), the presence of which is obvious from the upperside when the size of the costal red spot is reduced compared to its extent on the underside - this character is of importance in the *romanovi* group, because it separates complexes of subspecies (col. pl. 5). In contrast, that character seems infrasubspecific in the *deckerti* group, as it is observed to vary within individual populations. In representatives of the nominate subspecies the size of the HW reddish spots is always the same on both wings surfaces.

The blackish submarginal band is always more or less widened in the *deckerti* and *charltonius* groups, but in the *romanovi* group, the costal part of this band is narrower than other bands. However, this character is not general: in the most north-western subspecies (*platon* SOCHIVKO, 2011, *ljudmilae* LESIN & KAABAK, 1991), this band is similar to that of *deckerti* specimens. At the opposite, representatives of taxa distributed close to the range of the *deckerti* group always have a reduced black pattern and this affects especially the submarginal band (this is particularly the case for *kabiri* EISNER & NAUMANN and *vaporosus* Av., but even *aenigma* DUBATOLOV & MILKO has this band reduced, while other elements of the black pattern are very well expressed).

It is worth to add that real *deckerti* taxa have a reduced HW underside suffusion in contrast to the expressed submarginal pattern - this is especially obvious in populations from the northern and central parts of the range, where even the black discal spot is usually moderately or strongly reduced. In *romanovi* taxa, the underside pattern is well developed as a rule - even the most whitened *alraschid* CHURKIN & PLETNEV has an obvious discal spot and extended suffusion. Finally, the same is true of taxa from the southern border of the *charltonius* area - i.e. in the *eisnerianus*-complex.

3. External characters in the *romanovi* group and microevolution

As we noted above, *romanovi* taxa show valuable distinctive characters that seem not so important for other groups. Detailed analyses of the colouration of known subspecies of the group were published by CHURKIN (2009) and CHURKIN & PLETNEV (2012), these analyses being focused on subspecific features.

The known subspecies can be arranged into different complexes depending on which distinguishing criteria are selected. In several subspecies the costal red spot has the same size on the upperside and underside of the HW: these are *romanovi* GR.-GR., *vaporosus* Av., *kabiri* EISNER & NAUMANN, *ljudmilae* LESIN & KAABAK, and *sochivkoi* CHURKIN. In addition, *aenigma* DUBATOLOV & MILKO must be joined to this group, but this taxon often has a reduced bigger red spot on the HW upperside. It is worth to note that only *romanovi* GR.-GR. demonstrates some small variability in the size of the costal spot. The other complex includes taxa with a reduced costal spot on the upperside of the HW (i.e. with an “additional blackish line”): these are *eugenia* CHURKIN, *marusya* CHURKIN & PLETNEV, *platon* SOCHIVKO, *varvara* CHURKIN, and *alraschid* CHURKIN & PLETNEV (pl. 6). The geographic distribution of these two complexes exhibits a complicated, mosaic structure.

♀ of three taxa often have an additional reddish spot between veins M3 and Cu1 on the HW upperside: in *romanovi* GR.-GR., *vaporosus* Av., and *kabiri* EISNER & NAUMANN, small specimens often have this spot more or less reduced, yet obvious. Such a small additional red spot is very rarely registered in the remaining subspecies of the *romanovi* group. However, what gives weight to this feature is that it is absent as a rule in all the other groups. The first author proposed that *romanovi* GR.-GR. and *kabiri* EISNER & NAUMANN/*vaporosus* Av. are closely related and had a common ancestor, the range of which was subsequently divided, with *romanovi* GR.-GR. originating by mixing with the whitish subspecies that lived in the Alai valley. This hypothesis would explain the dubious position of that subspecies in the structure of the group in general, and the existence of genuine connections with both the *kabiri-vaporosus* branch and the *eugenia-marusya* branch. Moreover, it implies that *romanovi* GR.-GR. constitutes the most heterogeneous taxon, which is objectively confirmed by its great variability and the long list of known forms/aberrations.

Only two taxa - *ljudmilae* LESIN & KAABAK and *platon* SOCHIVKO - have a widened submarginal band (similar to that of the nominate subspecies, for example). Zoogeographically, both taxa inhabit Ghissar; moreover, SOCHIVKO found some populations with intermediate characters which populate the geographically intermediate zone near Anzob Pass and Iskanderkul Lake (A. SOCHIVKO, pers. comm.) which lies between the known distribution areas of the two taxa. Thus, we are led to conclude that these taxa originated from a common ancestor.

Summarizing the situation with the “*romanovi-eugenia*” and “*ljudmilae-platon*” cases, we can state that reduction of the size of the red spots on the upperside is likely to have appeared independently in the different subspecific branches, so that the weight of this character cannot be more than subspecific. This is consistent with the situation in the *deckerti* group, where this character has no serious signification at all, being infrasubspecific. We surmise that in the *romanovi* group, this subspecific character was incorporated along with the process that gave birth to some subspecies due to the pressure imposed by natural selection (see below).

In two cases one subspecies belonging to the *romanovi* group lives very close to another one and these cases seem to be very important.

Parnassius ch. alraschid CHURKIN & PLETNEV is very local and inhabits several very small localities located along the Gulcha river, in North-East Alai; *sochivkoi* CHURKIN populates several big river valleys in north Alai, but its type locality, along the upper course of the Ak-Bura river, lies 20-30 km from the type locality of *alraschid* CHURKIN & PLETNEV. The altitudes at which these two taxa are found can vary, but *sochivkoi* CHURKIN prefers rocks at higher elevations, whereas *alraschid* CHURKIN & PLETNEV, which lives lower on dry limestone cliffs, flies significantly later (by 7-10 days - 3 years of observations - statistically). The distribution areas of the two taxa are separated by a high mountain spur (which is currently free of glaciers), but we cannot exclude that *sochivkoi* CHURKIN will eventually be found in upstream Gulcha - there is no obvious reason to exclude this possibility. The two taxa use different foodplants, but it is known that one subspecies of gliding *Parnassius* can use different *Corydalis* species in different parts of its distribution area without any problems. The differences between *sochivkoi* CHURKIN and *alraschid* CHURKIN & PLETNEV are serious and constant, it is not possible to confuse any actual specimen. However, 1 ♂ with mixed characters, was recorded, hinting at the possibility of hybridization (CHURKIN & PLETNEV, 2012).

Another case of close contact is known from the Obikhingou river basin, in Tadjikistan. Two populations belonging to the *romanovi* group were found there in 2011 (details in CHURKIN & PLETNEV, 2014): they are *marusya* CHURKIN & PLETNEV on the one hand and a high mountain population of *vaporosus* Av. on the other. The two places, which are part of a continuous slope of the Mazorsky range, are separated by less than 15 km, without any intervening barriers; *vaporosus* Av. flies much higher than *marusya* CHURKIN & PLETNEV, but two weeks (!) earlier. The two taxa differ not only ecologically, but also morphologically in such an extent (col. pl. 7), that they would have been granted specific status had they belonged to Lycaenidae or Satyridae. However, hybridization is documented (col. pl. 2), even though the abundance and/or fertility of hybrids must be insufficient to ensure fusion of the two taxa (it is worth noting that the three biggest and most colourful *vaporosus* Av. specimens collected from that area have asymmetric or deformed wings, or even deformed genitalia). North Darwas represents the border between the distribution areas of the *vaporosus* complex (*vaporosus* Av. and *kabiri* EISNER & NAUMANN) populate all of the territory of the West Pamirs and Afghan Badakhshan) and another complex that groups together the taxa feeding on *Corydalis pseudoadunca* and living along river banks: *marusya* CHURKIN & PLETNEV, *eugenia* CHURKIN, *alraschid* CHURKIN & PLETNEV, *varvara* CHURKIN, and *aenigma* DUBATOLOV & MILKO.

We speculate that some additional cases of quasi-cohabitation of distinct *romanovi* taxa will be found in the future.

Thus, this group is seen to consist of several essentially different complexes between which hybridization is not straightforward. Such a structure, which is dramatically different from the known structure of other *P. charltonius* subspecific groups, betrays the existence of peculiarities specific to the natural history and evolution of the *romanovi* group.

4. Genitalia

♂ and ♀ genitalia of *Kailasius* or *Koramius* are very similar and do not exhibit any serious differences, except for the genitalia of *P. charltonius* GRAY. As stated by CHURKIN (2006), the ♂ genitalia of *P. charltonius* GRAY differs dramatically from those of the other species by the structure and position of the valvae, while the parts of the uncus are not widely separated, but practically joined together, so that they work as one united system; all these characters are apomorphic. It should be recalled that, with respect to the taxonomy and evolution of *Parnassius*, the uncus constitutes the most valuable of the sclerites. Moreover, the unusual snail-shaped sphragis of *charltonius* ♀♀ is unique among *Parnassius*. At the same time, there is no doubt (and genetically confirmed) that *P. charltonius* GRAY. is closely related to the rest of *Kailasius* - thus, we can conclude that the extent of evolution undergone by the genitalia of its ancestor since its separation from other members of the genus was both quite significant and unusually rapid.

We have not yet studied in details the sphragis and the ♀ genitalia; it can readily be noted that *aenigma* DUBATOLOV & MILKO and some related taxa have a very small sphragis compared to that of *deckerti* ♀♀. However, distinctions between the different groups or complexes seem to be not so simple and (this is more important) not so useful in taxonomic practice.

The ♂ genitalia represent the opposite case, since they demonstrate strikingly distinctive features. We performed more than 90 dissections, a total which includes all *romanovi* taxa and more than 20 representatives of the *deckerti* group, but only 2 ♂♂ of *voighti* BANG-HAAS and two ♂♂ of *charltonius* GRAY.

The individual variability of the ♂ genitalia was studied in a population of *kabiri* EISNER & NAUMANN (Vanch Mts, 20 dissections) and in both known populations of *anjuta* SHCHETKIN & KAABAK (12 dissections). It was found out that individual variability is not high; moreover, the size of the genitalia is more or less constant and does not depend strongly on the size of the specimen.

Serious differences between the genitalia of representatives of the *romanovi* and *deckerti* groups were first reported during a comparison between *aenigma* DUBATOLOV & MILKO and *anjuta* SHCHETKIN & KAABAK (CHURKIN & PLETNEV, 2012). Our current study confirms that all published distinctions are constant for all studied representatives of the two groups. ♂♂ of the *romanovi* group have a small uncus (usually devoid of teeth, but some small teeth are rarely present), the total length of which is obviously shorter than the length of tegumen. The ♂♂ of the *deckerti* group (including southern taxa like *amabilis* BRYK & EISNER, 1932 or *serenissimus* BRYK, 1932) have a very large uncus (usually with big teeth), the length of which is obviously much longer than the length of the reduced tegumen (pl. 10, 11). The differences easily can be seen from the lateral side (pl. 10, upper row) as well as from the dorsal or ventral sides (pl. 12, 13). The general shape of the uncus is similar in the two groups, and this must be the reason why nobody noted such an important feature before. The valvae of the two groups are about the same size and are typically, the same is true of the harpe. As a result, the abrupt differences in the size of the uncus and tegumen result in two different “mechanical” systems: there is no doubt that copulations between representatives of the two groups of taxa would be complicated or impossible. From a dorsal view, the long and massive *deckerti* uncus protrudes anteriorly compared to the ends of harpae, while in the *romanovi* group, the uncus ends at the same level as the distal extremity of the harpae.

The valva of the *romanovi* group is obviously rectangular, moderately long, while in the *deckerti* group, the valva is typically wide and gradually tapering towards the end. The harpe is more or less similar in both taxa, but the juxta (directly involved into the work of the valvae and aedeagus during copulation) is definitely different: it is big and well sclerotized in *deckerti*, but weak in the *romanovi* type. However, the degree of sclerotization of the juxta depends on the particular method and duration of preparation. More important is the fact that the juxta provides another very simple and obvious taxonomic feature: it is fully visible in all *deckerti* taxa from the dorsal side because the tegumen is short, while in the *romanovi* taxa, the juxta is practically not visible from the dorsal side because it is covered (enclosed) by the large tegumen (pl. 10, 11, 14).

The aedeagus of the *romanovi* group can be easily differentiated from that of the *deckerti* taxa because it is obviously widened at the distal end. This character is also absolutely constant, and it is easy to perform quick unambiguous identification of a specimen using the aedeagus only (plate 8, 9).

We did not find any serious variability in the *deckerti* complex, the genitalia of the butterflies from East Pamirs seem to be identical to those of specimens collected in the Himalayas.

In contrast, important variations were found in the *romanovi* group. Three taxa have a slightly more developed uncus: these are *romanovi* GR.-GR. and especially *kabiri* EISNER & NAUMANN/*vaporosus* AV. The general structure of the genitalia is identical, but an experienced scientist is able to identify and set apart the specimens of these subspecies. Unfortunately, these differences are not so obvious on the photographs, because the relative length of the harpae and uncus is sensitive to the small deformations of the prepared genitalia (those always exists) and - this is more important

- to small differences in the angle of vision (shooting). In total, the uncus is typically 10-15 % longer in this complex than in other *romanovi* taxa. Interestingly, *romanovi* ♂♂ sometimes have shorter and more massive valvae. Compared with all other taxa, *aenigma* DUBATOLOV & MILKO, *varvara* CHURKIN, *platon* SOCHIVKO, and *ljudmilae* LESIN & KAABAK have the smallest uncus.

In the nominate subspecies (*charltonius* GRAY) the genitalia are very similar to those of the *deckerti* group (pl. 10, 12), the uncus is not as massive, but very long, the valva and juxta seem identical, the aedeagus shows no distinctive widening at the end, but is obviously smaller (pl. 8, 9).

The *voigti* group (pl. 10, 12) is characterized by genitalia very similar to those of the *romanovi* group, the uncus is even smaller than in *aenigma* DUBATOLOV & MILKO or *ljudmilae* LESIN & KAABAK. In consequence, the uncus, when compared to that of the neighbouring taxon of the *romanovi* group (*kabiri* EISNER & NAUMANN), looks obviously smaller, but this difference is not as crucial as that between the *romanovi* and *deckerti* groups and should not result in copulative isolation. At the same time, the shape of the valva is the same as in the *deckerti* group (clearly shorter than in the *romanovi* group and not rectangular). The aedeagus has the same structure as in the *romanovi* group, but distal widening is not as clearly expressed (pl. 8, 9).

In summary, the *voigti* group does not differ from other taxa as drastically as the *romanovi*-group. However, its set of characters (including colouration) seems specific. We also cannot exclude that some distinguishing features were missed because the number of dissections was insufficient. The same is true for *charltonius* GRAY. The differences between the general structure of the '*romanovi*+*voigti*' and '*deckerti*+*charltonius*' genitalia are so deep and abrupt, that smaller specific characters could have been masked. It is necessary to carry out many additional dissections of *voigti* BANG-HAAS and *charltonius* GRAY ♂♂ to solve this problem.

II. Study of mtDNA

1. Materials and Methods - DNA extraction, amplification and sequencing

Specimens from which DNA was successfully amplified are listed in table 1. DNA was extracted from a single dried leg, either as described in AUBERT et al. (1999) or by using the Nucleo Spin Tissue XS kit from MACHEREY-NAGEL; both procedures were found to work with a reasonably high rate of success (at least 50 percent) for specimens collected up to 40 years ago (see fig. 1 in MICHEL et al., 2008).

PCR amplification of the COI (barcoding) mitochondrial segment was carried out as described in MICHEL et al. (2008) with primers 21686 (5'- ATTCAACAAATCATAAAGATATTGG) and COI-2192 (5'- CCCGGTAAAATTAAAA-TATAAACTTC), which generate a 727 nt DNA product. If necessary (e.g. in case of a partially degraded sample), these primers were combined with primers 21930 (5'-AGTTCCTGTCYCCATTTC) and 22014 (5'- GAAAATG-GRGCAGGAAC), respectively, to generate shorter products (350 and 395 nt, respectively). PCR amplification of the ITS2 nuclear DNA segment was obtained as described by MICHEL et al. (2013), with primers ITS3B (5'- GGTC-GATGAAGAACGCAGTTA) and ITS4B (5'-TTTCCTCCGCTTACTAATATGCTTA) which were found to generate a DNA product of 682 to 689 nt (depending on the sample).

After gel extraction and reamplification of the PCR product, purified DNA was sequenced on both strands at GATC Biotech with the primers used for PCR. Any ambiguity was resolved by examining chromatograms with BioEdit version 7.0.9.0 (HALL, 1999). COI accession numbers are listed in table 1. ITS2 sequences were generated from the following five DNA extracts: *charltonius* GRAY W357 (accession # KJ958545), *anjuta* SHCHETKIN & KAABAK W355 (# KJ958546), *deckerti* VERITY W359 (# KJ958547), *ljudmilae* LESIN & KAABAK W361 (# KJ958548), *aenigma* DUBATOLOV & MILKO W363 (# KJ958549).

Phylogenetic analyses

Aligned positions 64 to 668 of the COI gene were used to build the median-joining network in fig. F2 with program Network (Fluxus Technology) version 4.6.1.1 (BANDELT et al., 1999). The phylogenetic tree in fig. F3 was generated with PAUP* 4.0b10 (SWOFFORD, 2002). The Neighbor-joining algorithm was used together with the LogDet (LOCKHART et al., 1994) measure of distance; the latter analysis makes it possible to include missing data, so that a somewhat larger expanse of sequence (aligned positions 39 to 687 of the COI gene) could be included. COI sequences of other members of the *Kailasius* subgenus (*P. inopinatus* KOTZSCH, *P. autocrator* AV., *P. loxias* PUENGELER, *P. davydovi* CHURKIN; MICHEL et al., 2008 and our own unpublished data; CONDAMINE et al., 2012) were used as outgroup, while the COI sequence of individual AC1-7 (CONDAMINE et al., 2012) was added to the ingroup. Bootstrap percentages were obtained from 1000 bootstrap replicates.

Alignment of the five ITS2 sequences over 585 positions required one single-nucleotide and four 2-nt indels. The latter were counted as single substitution events when computing the distance matrix in Table II.

2. Results

The 24 individuals of *P. charltonius* GRAY whose DNA was analyzed in this work (table 1) were selected in order to sample the known range of *P. charltonius* GRAY as exhaustively as possible (Fig. F1). Although necessarily sparse, given the geographic expanse to be covered, our sampling was reasonably even, except at the eastern edges of the range; from these parts, few specimens are available and most of them were collected quite long ago.

Mitochondrial genotypes ('haplotypes', since mitochondrial DNA is clonally inherited, from the mother) were obtained by sequencing the very same COI segment that has become widely used to 'barcode' animals (HEBERT et al., 2003). Mitochondrial genetic diversity within *P. charltonius* GRAY was found to be moderate, amounting to 2.8 % at most (18 substitutions out of 641 aligned nucleotides), between individuals W357 (*P. ch. charltonius* GRAY) and W396 (*P. ch. flau-geri* EISNER). However, the distribution of haplotypes in sequence space is quite striking. In the median-joining network of Fig. F2, 23 of the 25 available sequences fall into two well separated clusters, whereas the remaining two haplotypes (W357 and W385), which come from the southwestern and southeastern edges of the *charltonius* range, are clear outliers. The two major genetic clusters, whose average genetic distance is 1.6 % (individual values range from 0.9 to 2.5 %), originate from separate geographic areas (fig. F1) and correspond to distinct morphological entities, which may conveniently be designated by the oldest named taxon in each subdivision, i.e. the *romanovi* group and the *deckerti* group. Median-joining networks are based on the assumption that evolution proceeds with maximum parsimony. Although the phylogenetic tree in fig. F3 was built by a different method, from a distance matrix, the outcome was essentially the same: in the *P. charltonius* GRAY subtree, *P. ch. charltonius* GRAY (W357) and *P. ch. voighti* BANG-HAAS (W385) haplotypes occupy basal positions, while the *deckerti* and *romanovi* groups constitute well separated clades, one of which at least (*deckerti*) is statistically supported by a reasonably high bootstrap percentage (78 %). Within the *romanovi* clade, some subgroups are strongly supported as well: *P. ch. aenigma* DUBATOLOV & MILKO (W363), *P. ch. varvara* CHURKIN (W377) and *P. ch. alraschid* CHURKIN & PLETNEV (W390), from the northeastern end of the range, are closely related and the same is true of *P. ch. platon* SOCHIVKO (W391) and *P. ch. ljudmilae* LESIN & KAABAK (W361), at the northwestern edge. Finally, *P. ch. eugenia* CHURKIN (W379) and *P. ch. marusya* CHURKIN & PLETNEV (W388) were found to possess identical COI haplotypes. The latter was collected only 12 km from individual W387 (*P. ch. vaporosus* Av.), which differs at four nucleotide positions and belongs to a morphologically distinct population. As for differences between the network in fig. F2 and the tree in fig. F3, they stem primarily from statistical uncertainty. Thus, while fig. F3 might be thought to suggest some specific relationship between the *P. romanovi* Gr.-Gr. W393 specimen and the *marusya-eugenia* branch, the bootstrap value for that particular node/subtree (33 %) is far too low for the association to be significant. Similarly, the location of that same W293 individual in the fig. F2 network, halfway between *P. ch. kabiri* EISNER & NAUMANN and *P. ch. eugenia* CHURKIN/*P. ch. marusya* CHURKIN & PLETNEV (two nucleotide substitutions away from each), does not mean that it is genetically intermediate - a meaningless statement, since mitochondrial DNA is inherited uniparentally, without recombination (MAYNARD-SMITH & SMITH, 2002) - but that the length of sequence available is insufficient to establish whether it belongs to the former or latter subset.

We wished to confirm our findings by examining nuclear DNA, whose inheritance is biparental, instead of uniparental. The ITS2 segment is part of the genetic unit that specifies ribosomal RNA and as such, is repeated hundreds of times in the genome, which makes its amplification feasible even from dried, (moderately) aged material. We succeeded in obtaining sequences from individual W357 (*P. ch. charltonius* GRAY) as well as two representatives of each of the two major clades. As seen in table II, nucleotide substitutions were even fewer than for the COI segment, which precludes statistical analysis. Still, we believe it worth noting that genetic distances within the *romanovi* and *deckerti* clades are smaller than those between the two clades, as had already been observed with mitochondrial haplotypes.

III. Discussion

Taxonomic systems that rest exclusively either on morphology or on the DNA sequences of a limited number of genes are bound to misrepresent the complexity of evolutionary processes. In what follows, we attempt a synthesis of our molecular, mtDNA data on the one hand, and morphological analyses on the other in order to try and build a coherent system that agrees with zoogeography. As will be emphasized, our data do not fit well the old typological conception, whereas making use of the modern biological species concept allowed us to provide consistent explanations for all available facts.

Our most striking finding is the presence of constant, major differences in the ♂ genitalia of two entities - *romanovi* and *deckerti* - that occupy together more than four-fifths of the geographic range of what had been regarded up to now as a single species, *P. charltonius* GRAY (fig. F1). There is no question that the *romanovi* and *deckerti* groups constitute distinct species as these two entities exhibit constant differences in wing pattern (this work) and, most important, possess not only distinct, but well separated sets of mitochondrial DNA genotypes (figs. F2 and F3). While our analysis of nuclear DNA remains far too preliminary to rule out the existence of some gene flow between these two newly recognized species in the Pamir region, where their ranges could meet (fig. F1), we regard that as an unlikely possibility given the magnitude of their genitalic incompatibility.

The nomenclatural consequences of the subdivision of *P. charltonius* GRAY into (at least) two species will depend on the actual status of the other two major branches revealed by our analysis of mitochondrial DNA (figs. F2 and F3). Despite its rather considerable genetic divergence from *deckerti* (as judged from the single sequenced individual), we failed to find serious morphological differences between *P. ch. charltonius* GRAY - only two specimens of which could be examined - and the *deckerti* group. We regard the status of *P. ch. charltonius* GRAY populations as an open problem, that a future, detailed study of all the taxa currently united under *deckerti* and *P. charltonius* GRAY may be able to solve. The correct status of the *voighti* group is especially problematic. The only specimen whose mitochondrial DNA could

be sequenced was found to be divergent from both, the *deckerti* and *romanovi* groups. Morphologically, the *voigti* group is characterized by a specific combination of characters. The wing pattern is basically similar to that of the *charltonius* and *deckerti* groups (the third submarginal spot on the HW underside is not seriously reduced), but the genitalia belong to the *romanovi* type, while submarginal HW spots are not contrasted and expressed. In addition, the black pattern is reduced and ♂♂ lack the anal red spot on the HW upperside. Geographically, the distances that separate known *voigti* populations of *P. ch. wernickei* KOTZSCH from neighboring populations of *P. ch. ducalis* BOULLET & LE CERF BOULLET & LE CERF (*deckerti* group) or *P. ch. kabiri* EISNER & NAUMANN (*romanovi* group) are quite small, yet hybrids have never been reported. Still, we regard it as premature to reach a decision on the status of this group until a detailed study of its various component taxa has been carried out. Unfortunately, material from Afghanistan is scarce and this situation is likely to persist.

How old is the separation of the *romanovi* group from the *deckerti* group? The time of divergence of the *romanovi* and *deckerti* mitochondrial clades may be roughly estimated from the average divergence of COI sequences (1.6%). This genetic distance is similar to the average distance, over the same COI barcoding segment, between members of the *P. (phoebus) phoebus* and *P. (phoebus) smintheus* lineages (1.9%, calculated from sequences published by MICHEL et al., 2008), the divergence of which was estimated to have occurred between 0.8 and 1.5 million years ago by TODISCO et al. (2012).

The various taxa that constitute the *romanovi* and *deckerti* groups are of course much younger (fig. F3). Still, our analyses reveal a significant extent of differentiation within both these entities. As shown by morphological analyses, and confirmed to some extent by the distribution of mitochondrial genotypes (see especially fig. F2), the *romanovi* group consists of two distinctively different complexes: *vaporosus* (which includes *P. ch. vaporosus* Av. itself and *P. ch. kabiri* EISNER & NAUMANN) and a second complex that unites all the other taxa, with the possible exception of *P. ch. romanovi* GR.-GR., the position of which remains dubious (see above). The latter complex originated at the north-western edges of the Asian mountain system and subdivided subsequently into several branches. Interestingly, members of one branch may at present live very close either to members of another branch or representatives of the *vaporosus* complex.

Parnassius ch. sochivkoi CHURKIN was treated as a synonym of *P. ch. romanovi* GR.-GR. (ROSE & WEISS, 2011). This decision, which was based on the external similarity of these taxa, ignored several somewhat 'smaller', but taxonomically valuable characters. According to our mtDNA analyses, *P. ch. romanovi* GR.-GR. and *P. ch. sochivkoi* CHURKIN appear to belong to different branches of the *romanovi* phylogenetic tree, which confirms the status of the latter taxon, as well as the usefulness of the system of characters highlighted in this work.

Parnassius ch. aenigma DUBATOLOV & MILKO, *P. ch. varvara* CHURKIN, and *P. ch. alraschid* CHURKIN & PLETNEV constitute three well differentiated subspecies, it is impossible to misidentify any specimen belonging to either one of these taxa (col. pl. 6). Undoubtedly, the main features of each taxon are genetically based (not ecologically), for the variability of diagnostic characters is practically nil (CHURKIN, 2009, CHURKIN & PLETNEV, 2012). These three subspecies, which occupy the northeastern extremity of the range of *P. romanovi* GR.-GR., are clearly related according to mtDNA data and their divergence was relatively recent. We propose that all three of them originated through the demographic bottleneck effect, a well known factor of evolution which results in inbreeding, genetic drift, and a high speed of evolutionary change. The fact is, that we are dealing with small, very local populations, which are practically isolated from each other, and present abrupt hiatuses in external colouration. At least two of these subspecies live under very special conditions at the northern limits of the *P. romanovi* GR.-GR. distribution area. Interestingly, *P. ch. varvara* CHURKIN and *P. ch. alraschid* CHURKIN & PLETNEV possess a number of unusual or marginal features: *P. ch. varvara* CHURKIN has joined FW discal spots and a curved costal FW margin, *P. ch. alraschid* CHURKIN & PLETNEV has the most whitened upperside in conjunction with a strongly expressed blackish underside suffusion, etc. Such features are suggestive indeed of genetic drift, subsequent to bottleneck events.

Rapid divergence under conditions of random catastrophic events that wipe out from time to time most of the local populations is expected to result in the surviving lineages being adapted to small, distinct ecological niches, some of which may be located very close geographically, as is the case for *P. ch. alraschid* CHURKIN & PLETNEV and *P. ch. sochivkoi* CHURKIN. The latter taxa constitute two different lineages within one branch of the *romanovi* group, whereas the pair formed by *vaporosus* and *marusya* complexes representatives of two major branches. Had we not a full picture of the evolution of the group, *P. ch. vaporosus* Av. and *P. ch. marusya* CHURKIN & PLETNEV might have been attributed to different species, while in reality we are (most probably) dealing with "semi-species" whose hybridization in nature may be difficult, but should remain possible. Additionally, our data point to *P. ch. vaporosus* Av. being autochthonous to Pamirs-Darwas, whereas *P. ch. marusya* CHURKIN & PLETNEV must result from a relatively recent expansion of the ancestral *aenigma* complex, possibly in relation with the spreading of the main foodplant of the latter - *Corydalis pseudodunca* M. POP. This plant is distributed from Kashgaria and the Naryn valley to the Darwas district, and at least five subspecies of the *romanovi* group use it.

By extension, it is logical to assume that the strong pressures of selection and rapid evolution that have been, and continue to be at play in the diversification of *P. romanovi* GR.-GR. were responsible in the first place for the divergence of this species from the ancestral *P. charltonius* GRAY stock.

The *deckerti* group offers another example of a good agreement between different methodological approaches. Admittedly, our sampling is somewhat insufficient, both in terms of sequence length and number of individuals examined, to allow analyses at the level of population genetics. Still, our data hint at the possibility of different demographic histories for *P. ch. deckerti* VRTY. and *P. romanovi* GR.-GR. *Parnassius romanovi* GR.-GR. individuals that come from neighboring districts have closely related mitochondrial genotypes (except in the case of *P. ch. vaporosus* AV. W387 and *P. ch. marusya* CHURKIN & PLETNEV W388) and genetic distance tends to grow steadily with geographic distance (fig. F2). In contrast, *P. ch. deckerti* VRTY. shows signs of a more diversified history. *P. ch. anjuta* SHCHETKIN & KAABAK and other true *P. ch. deckerti* VRTY. taxa constitute a more or less homogeneous complex of populations, within which the short times of isolation implied by DNA sequences agree well with the absence of serious differences in genitalia or colouration. At the same time, genetic diversity is significant in Ladakh and neighboring districts, suggestive of a stable, sizeable genetic pool (compare individuals W365, W375, W359 and W407 in fig. F2; the latter two belong to the same subspecies). Only at the edge of the *P. ch. deckerti* VRTY. range, do specimens W401, W399, W405 and W355, which are spread over an arc of close to 500 km, have identical or nearly identical genotypes, pointing to a recent, presumably postglacial geographic expansion. Thus, the hypothesis that *P. ch. anjuta* SHCHETKIN & KAABAK, which lives in high altitude, dry environments quite similar to those of *P. ch. deckerti* VRTY., results from a recent penetration of the East Pamirian Plateau (CHURKIN & PLETNEV, 2012) has now been proven by molecular data. And despite the current lack of sequence data, it is logical as well to assume that representatives of *P. ch. deckerti* VRTY. occupied the northern slopes of Karakorum only recently (as noted above for *P. ch. mazhaensis* HUANG). Noteworthy, some small, but more or less constant differences between real *P. ch. anjuta* SHCHETKIN & KAABAK and the local form known as “*mistericus*” could be interpreted as resulting again from bottleneck events, since the latter should be frequent under the severe conditions of the East Pamirs.

It should also be emphasized that not only *P. romanovi* GR.-GR. (whose individual populations occur at very different altitudes and have markedly distinct flight periods (CHURKIN, 2009), but also *P. ch. deckerti* VRTY. has undergone considerable ecological diversification. In contrast to morphologically alike *P. ch. anjuta* SHCHETKIN & KAABAK and *P. ch. deckerti* VRTY., which despite being separated by some 500 km, have similar, very dry habitats in the inner mountains ranges of Pamir and Himalaya, respectively, *P. ch. flaugeri* EISNER and *P. ch. serenissimus* BRYK (the latter flies hardly more than 100 km away from *P. ch. deckerti* VRTY.) inhabit monsoon-drenched slopes and ridges and their larvae, which are quite distinct morphologically from those of *P. ch. deckerti* VRTY. (MICHEL & WEISS, unpublished observations), feed on soft-leaved *Corydalis* sp., instead of the very tough foliage of *C. flabellata*, which is utilized as a foodplant by the *P. ch. deckerti* VRTY. populations of Ladakh. Somewhat surprisingly, however, the life cycle seems pretty uniform throughout what had been regarded up to now as *P. charltonius* GRAY: it was argued by CHURKIN (2009) that all populations of the *romanovi* group have a two-year cycle, and a 2-year cycle as well, with both egg and pupal diapause, was observed in the laboratory by one of us (F.M.) for *P. ch. flaugeri* EISNER of the *deckerti* group. In individual populations, adults may be much more frequent of course on odd or even years, but this phenomenon has generally not been found to prevent genetic exchange in other mountain butterflies (see VILA & BJÖRKLUND, 2004, and references therein).

Finally, we would like to mention that we had the opportunity to discuss our analyses with well-known evolutionist ALEXEY S. KONDRASHOV (Moscow State University, University of Michigan), who confirmed our conclusions, especially about the importance of bottleneck effects, and informed us of similar findings for different Vertebrata as well as for some laboratory insects (for example, *Drosophila* sp.).

Conclusions

1. *Parnassius romanovi* GRUM-GRSHIMAILO, 1885 **stat. nov.** constitutes a bona species distributed in Alai, Ghissar, West Pamirs/Badakhshan and the SW edges of Tian-Shan. It is characterized by a reduced uncus (which is shorter than the tegumen) and a reduced third submarginal spot on the HW underside. This species consists of two divergent complexes of subspecies: the *romanovi* complex (*P. romanovi romanovi* GR.-GR., 1885 **stat. nov.** - more information about the position of this taxon see in the Discussion, *P. r. ljudmilae* LESIN & KAABAK, 1991 **comb. nov.**, *P. r. sochivkoi* CHURKIN, 2009 **comb. nov.**, *P. r. aenigma* DUBATOLOV & MILKO, 2003 **comb. nov.**, *P. r. eugenia* CHURKIN, 2009 **comb. nov.**, *P. r. marusya* CHURKIN & PLETNEV, 2012 **comb. nov.**, *P. r. platon* SOCHIVKO, 2011 **comb. nov.**, *P. r. varvara* CHURKIN, 2009 **comb. nov.**, *P. r. alraschid* CHURKIN & PLETNEV, 2012 **comb. nov.**), and the *vaporosus* complex (*P. r. vaporosus* AVINOV, 1913 **comb. nov.**, *P. r. kabiri* EISNER & NAUMANN, 1980 **comb. nov.**).
2. The status of *P. ch. deckerti* VERTY, 1907 and *P. ch. voigti* BANG-HAAS, 1927 is questionable and needs further clarification. Especially the latter taxon could constitute a separate species.
3. The comparative analysis of our mtDNA and morphological data makes it possible to interpret the action of evolutionary factors in the history of the taxa.

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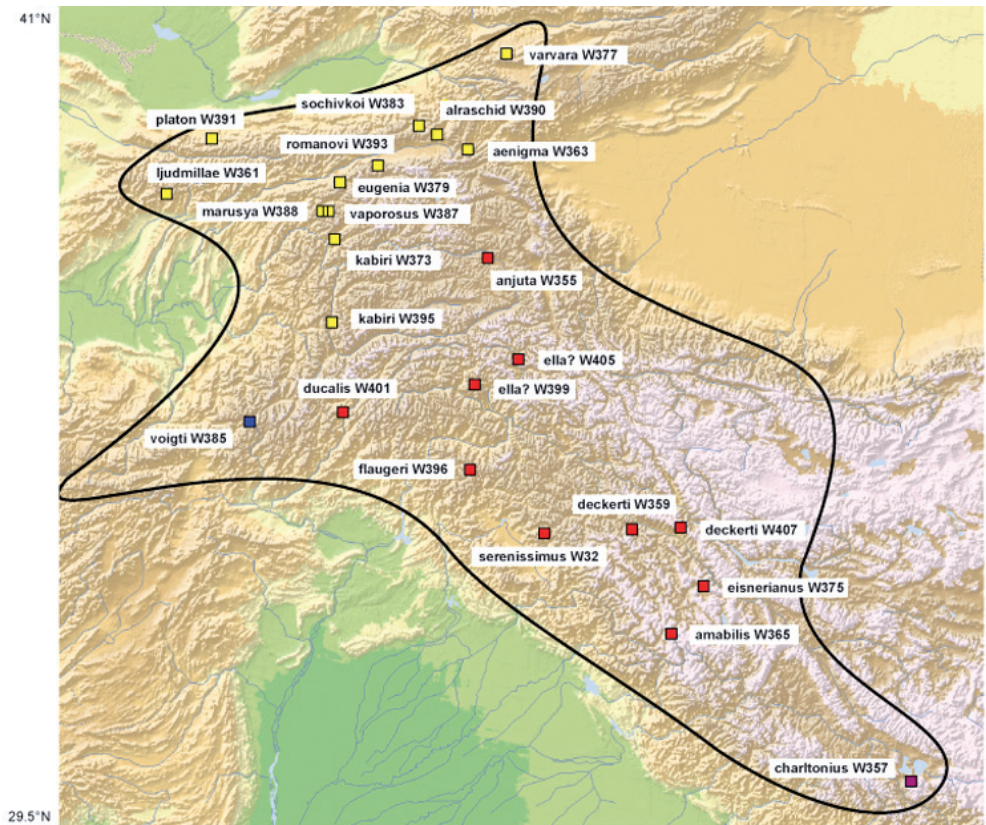


Figure F1: Geographic location of specimens used for DNA analyses. The thick black curve (slightly modified from ROSE & WEISS, 2011) delimits the approximate range of the *Parnassius charltonius* GRAY, 1853 complex. Sites of origin of mitochondrial haplotypes belonging to the *deckerti* clade are shown in red, those of the *romanovi* clade are in yellow.

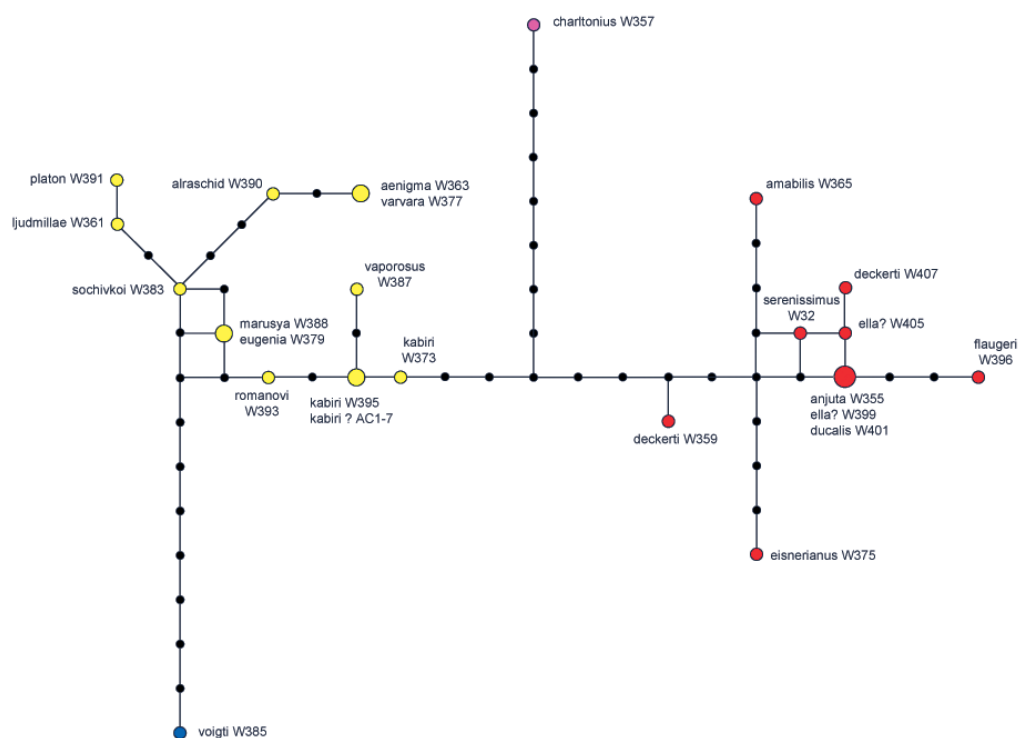


Figure F2: Median-joining network of COI haplotypes. A single nucleotide substitution separates a node from its immediate neighbor(s). Nodes/haplotypes that belong to the *deckerti* group are coloured in red, those of the *romanovi* group are in yellow, small black nodes correspond to inferred, possibly ancestral sequences. Note that the size of a coloured node is proportional to the number of haplotypes observed at that site. Numbers next to a node are bootstrap percentages (i.e. the percentage of trees, after bootstrap resampling of the original data, in which that particular subclade is observed); only values equal to, or greater than 50 are shown.

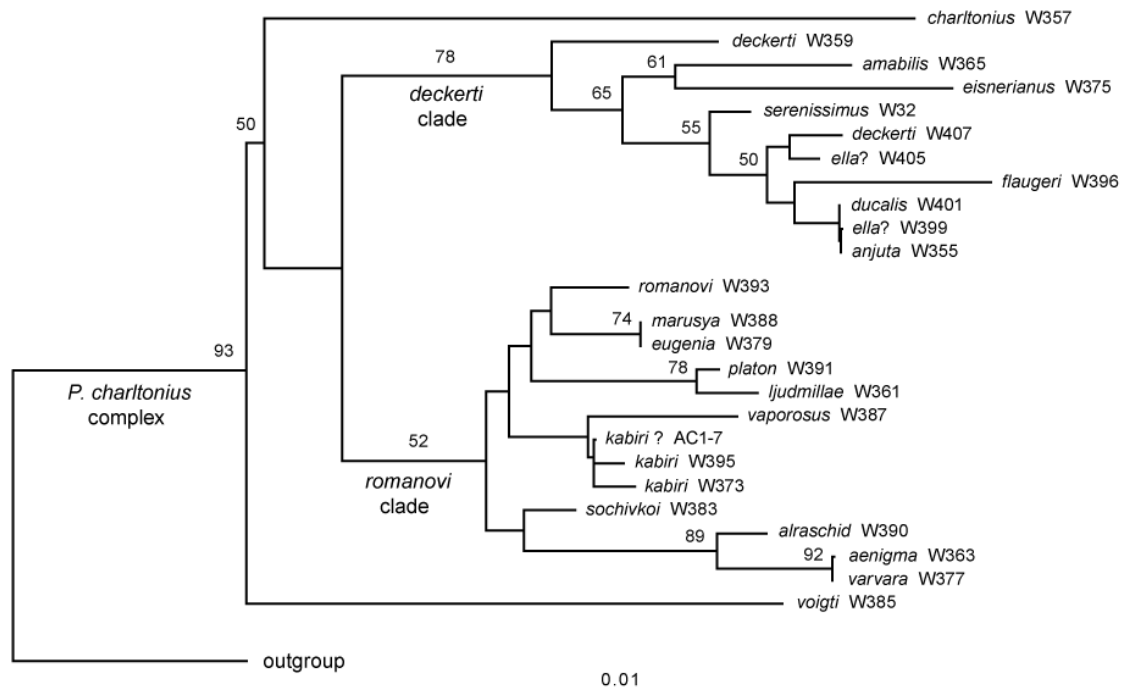


Figure F3: Phylogeny of mitochondrial genotypes. The tree was built by the Neighbour-Joining procedure with a LogDet measure of distance, using other taxa of *Parnassius* subgenus *Kailasius* as outgroup (see Materials and Methods).

Specimen	Taxon	Sex	Locality	Country	Sequence Accession #
W32	<i>P. charltonius serenissimus</i> BRYK, 1932	female	S of Sonamarg (4000 m), Kashmir	India	AM231451
W355	<i>P. charltonius anjuta</i> SHCHETKIN & KAABAK, 1985	male	Mynkhadzhir Mts., East Pamir	Tajikistan	KJ961616
W357	<i>P. charltonius charltonius</i> GRAY, [1853]	male	Mandhata Mt. (4800-5200 m), W Tibet	China	KJ961609
W359	<i>P. charltonius deckerti</i> VERITY, 1907	male	Lamayuru (3900 m), Ladakh	India	KJ961610
W361	<i>P. romanovi ljudmillae</i> LESIN & KAABAK, 1991	male (topotype)	Karatag R. basin (3500 m), Ghissar	Tajikistan	KJ961630
W363	<i>P. romanovi aenigma</i> DUBATOLOV & MILKO, 2003	male (topotype)	Irkeshtam, Chinese Kyzyl-Su river, Kyrgyzstan	Kyrgyzstan	KJ961627
W365	<i>P. charltonius amabilis</i> BRYK & EISNER, 1932	female	Bhaga valley, Baralacha La (4400 m)	India	KJ961611
W373	<i>P. romanovi kabiri</i> EISNER & NAUMANN, 1980	female	Gyshkhun (3000 m), Vanch Mts., W Pamir	Tajikistan	KJ961625
W375	<i>P. charltonius eisnerianus</i> BRYK, 1931	female	N of Tsokar (4600 m), Ladakh	India	KJ961612
W377	<i>P. romanovi varvara</i> CHURKIN, 2009	male 2** (holotype)	Karasu R. (3000 m), Dzhaman-Too Mts., Tian-Shan	Kyrgyzstan	KJ961628
W379	<i>P. romanovi eugenia</i> CHURKIN, 2009	male 3A** (holotype)	Muksu R., Peter I Mts.	Tajikistan	KJ961629
W383	<i>P. romanovi sochivkoi</i> CHURKIN, 2009	male 1A** (holotype)	Ak-Bura River (2600-2700 m), NE Alai	Kyrgyzstan	KJ961631
W385	<i>P. charltonius voighti</i> BANG-HAAS, 1927	male	Panjshir valley (3500-4000 m), Parvan prov.	Afghanistan	KJ961619
W387	<i>P. romanovi vaporosus</i> AVINOV, 1913	male	Obimazor (3500 m), Mazorsky Mts., Darvas	Tajikistan	KJ961622
W388	<i>P. romanovi marusya</i> CHURKIN & PLETNEV, 2012	female (paratype)	Obikhingou R. (2200-2400 m), Darvas	Tajikistan	KJ961623
W390	<i>P. romanovi alraschid</i> CHURKIN & PLETNEV, 2012	male (paratype)	Gulcha R. (2200-2500 m), East Alai	Kyrgyzstan	KJ961626
W391	<i>P. romanovi platon</i> SOCHIVKO & KAABAK, 2011	male (topotype)	Yangiaryk (1800-1900 m)	Tajikistan	KJ961621
W393	<i>P. romanovi romanovi</i> GRUM-GRSHIMAILO, 1885	female (topotype)	Aram Kungei R. (3500 m), Trans-Alai Mts.	Kyrgyzstan	KJ961620
W395	<i>P. romanovi kabiri</i> EISNER & NAUMANN, 1980	female	Kuh-i-Lal (3500 m), W Pamir	Tajikistan	KJ961624
W396	<i>P. charltonius flaugeri</i> EISNER, 1978	female	Babusar Pass	Pakistan	KJ961614
W399	<i>P. charltonius deckerti</i> VERITY, 1907 (ella BRYK, 1932 ?)*	male	Phakora to Naltar (2000-3200 m), Gilgit	Pakistan	KJ961615
W401	<i>P. charltonius ducalis</i> BOULLET & LE CERE, 1912	male	Birmoglasht (2500-4000 m), Chitral	Pakistan	KJ961618
W405	<i>P. charltonius deckerti</i> VERITY, 1907 (ella BRYK, 1932 ?)*	male	Soost (3000-4000 m), Khudabaad Mts.	Pakistan	KJ961617
W407	<i>P. charltonius deckerti</i> VERITY, 1907	male	S of Khardung La (5000 m), Ladakh	India	KJ961613

Table I: Samples used for mitDNA analyses

* The status and relationships of the taxon „ella“ are questionable; the studied populations belong to the *deckerti* complex.

** Numbers are those of individuals in the original description plates.

	W357	W355	W359	W361	W363
<i>charltonius</i> W357	-	15	12	13	16
<i>anjuta</i> W355	4	5	5	12	11
<i>deckerti</i> W359	7	3	9	9	10
<i>ljudmillae</i> W361	8	6	9	2	7
<i>aenigma</i> W363	8	6	9	2	-

Table II : Comparison of the numbers of substitutions over mitochondrial DNA segment COI (above diagonal, 641 aligned positions) and nuclear DNA segment ITS2 (below diagonal, 585 aligned positions). The two smallest values in each half-table are circled.

Specimen	Taxon	Locality	Country
<i>anjuta</i>	<i>Parnassius charltonius anjuta</i> SHCHETKIN & KAABAK, 1985 (topotype)	Mynkhadzhayr Mts., E. Pamirs (1995)	Tajikistan
<i>anjuta -2</i>	<i>P. ch. anjuta</i> SHCHETKIN & KAABAK, 1985 (topotype)	Mynkhadzhayr Mts., E. Pamirs (1997)	Tajikistan
<i>mistericus</i>	<i>P. ch. anjuta</i> SHCHETKIN & KAABAK, 1985 (<i>mistericus</i> -topotype)	Dunkeldyk L., E. Pamirs (1995)	Tajikistan
<i>mistericus-2</i>	<i>P. ch. anjuta</i> SHCHETKIN & KAABAK, 1985 (<i>mistericus</i> -topotype)	Dunkeldyk L., E. Pamirs (1996)	Tajikistan
<i>deckerti</i>	<i>P. ch. deckerti</i> VERITY, 1907	Lamayuru, Ladakh (1997)	India
<i>ducalis</i>	<i>P. ch. ducalis</i> BOULLET & LE CERF, 1912	Shandur Pass (1996)	Pakistan
<i>eisnerianus</i>	<i>P. ch. eisnerianus</i> BRYK, 1931	Baralacha pass, Lahoul (1997)	India
<i>flaugeri</i>	<i>P. ch. flaugeri</i> EISNER, 1978	Junkar, Jammu&Kashmir (2003)	Pakistan
<i>charltonius</i>	<i>P. ch. charltonius</i> GRAY, [1853]	Mandhata Mt., W. Tibet (2006)	China
<i>charltonius-2</i>	<i>P. ch. charltonius</i> GRAY, [1853]	Mandhata Mt., W. Tibet (2006)	China
<i>voigti</i>	<i>P. ch. voigti</i> BANG-HAAS, 1927	Zanglah, Koh-e-Baba Mts. (2002)	Afghanistan
<i>voigti-2</i>	<i>P. ch. voigti</i> BANG-HAAS, 1927	Kotal-e-Shawa, Panjshir valley (1999)	Afghanistan
<i>voigti-3</i>	<i>P. ch. voigti</i> BANG-HAAS, 1927	Kotal-e-Shawa, Panjshir valley (1999)	Afghanistan
<i>aenigma</i>	<i>P. romanovi aenigma</i> DUBATOLOV & MILKO, 2003 (topotype)	Irkeshtam, Chinese Kyzyl-Su river (2005)	Kyrgyzstan
<i>alraschid</i>	<i>P. r. alraschid</i> CHURKIN & PLETNEV, 2012 (paratype)	Gulcha R., East Alai (2011)	Kyrgyzstan
<i>varvara</i>	<i>P. r. varvara</i> CHURKIN, 2009 (paratype)	Karasu R., Dzhaman-Too, Tian-Shan (2008)	Kyrgyzstan
<i>varvara -2</i>	<i>P. r. varvara</i> CHURKIN, 2009 (topotype)	Karasu R., Dzhaman-Too, Tian-Shan (2010)	Kyrgyzstan
<i>marusya</i>	<i>P. r. marusya</i> CHURKIN & PLETNEV, 2012 (paratype)	Obikhingou R., Darwas (2011)	Tajikistan
<i>eugenia</i>	<i>P. r. eugenia</i> CHURKIN, 2009 (paratype)	Muksu R., Peter I Mts. (2009)	Tajikistan
<i>eugenia -2</i>	<i>P. r. eugenia</i> CHURKIN, 2009 (topotype)	Muksu R., Peter I Mts. (2011)	Tajikistan
<i>platon</i>	<i>P. r. platon</i> SOCHIVKO & KAABAK, 2011 (topotype)	Yangiaryk, Turkestan sky Mts. (2011)	Tajikistan
<i>platon - 2</i>	<i>P. r. platon</i> SOCHIVKO & KAABAK, 2011 (paratype)	Sarkat River, Turkestan sky Mts. (2009)	Kyrgyzstan
<i>ljudmillae</i>	<i>P. r. ljudmillae</i> LESIN & KAABAK, 1991	Karatag R. basin, Ghissar (2011)	Tajikistan
<i>romanovi</i>	<i>P. r. romanovi</i> GRUM-GRSHIMAILO, 1885 (topotype)	Aram Kungei R., Trans-Alai Mts. (2004)	Kyrgyzstan
<i>vaporosus</i>	<i>P. r. vaporosus</i> AVINOV, 1913	Obimazor, Mazorsky Mts., Darwas (2011)	Tajikistan
<i>kabiri</i>	<i>P. r. kabiri</i> EISNER & NAUMANN, 1980 (topotype)	Shiva Lake, Badakhshan (2011)	Afghanistan
<i>kabiri - 2</i>	<i>P. r. kabiri</i> EISNER & NAUMANN, 1980	Gyshkhun, Vanch Mts., W. Pamir (2001)	Tajikistan
<i>kabiri - 3</i>	<i>P. r. kabiri</i> EISNER & NAUMANN, 1980	Sangou-Dara, Khorog, W. Pamir	Tajikistan
<i>kabiri - 4</i>	<i>P. r. kabiri</i> EISNER & NAUMANN, 1980	Gyshkhun, Vanch Mts., W. Pamir (2003)	Tajikistan
<i>sochivkoi</i>	<i>P. r. sochivkoi</i> CHURKIN, 2009 (paratype)	Ak-Bura R., NE Alai (2009)	Kyrgyzstan

Table III. Samples used for the genitalia and wings analyses (plates 2-5, 8-14; in the plates the ♀♀ are marked with the special sign, otherwise the ♂♂ (or their parts) are shown; the collecting data are the same for ♂♂ and ♀♀).



Plate 1. *Parnassius* species, ♂♂: 1, 2 - *P. charltonius flaugeri* EISNER, 1978; 3 - *P. ch. ducalis* BOULLET & LE CERF, 1912, 4 - *P. ch. deckerti* VERITY, 1907, 5 - *P. ch. anjuta* SHCHETKIN & KAABAK, 1985, 6 - *P. romanovi romanovi* GRUM-GRSHIMAILO, 1885 **stat. nov.**, 7 - *P. r. aenigma* DUBATOLOV & MILKO, 2003 **comb. nov.**, 8 - *P. r. sochivkoi* CHURKIN, 2009 **comb. nov.**, 9 - *P. r. kabiri* EISNER & NAUMANN, 1980 **comb. nov.**, 10 - *P. r. ljudmilae* LESIN & KAABAK, 1991 **comb. nov.** (additional data - Table III).



Plate 2. *Parnassius* species: 1, 3 - *P. charltonius voighti* BANG-HAAS, 1927 (Panjshir vall., ♂, ♀); 2 - *P. ch. voighti* BANG-HAAS, 1927 (Koh-e-Baba, ♂); 4, 5, 6 - *P. ch. charltonius* GRAY, [1853] (Mandhata, ♂, 2 ♀♀); 7 - *P. romanovi marusya* CHURKIN & PLETNEV, 2012 **comb. nov.** (*kabiri*-like aberration, Obikhingou, paratype ♂); 8 - *P. r. marusya* CHURKIN & PLETNEV, 2012 **comb. nov.** (*anjuta*-like aberration, Obikhingou, paratype ♀); 9 - *P. r. vaporosus* AVINOV, 1912 **comb. nov.** (*romanovi*-like form, Mazorsky range, 2013, ♂); 10 - *P. r. kabiri* EISNER & NAUMANN, 1980 **comb. nov.** (aberration with *anjuta*- and *romanovi*-mixed characters, Vanch, ♂).

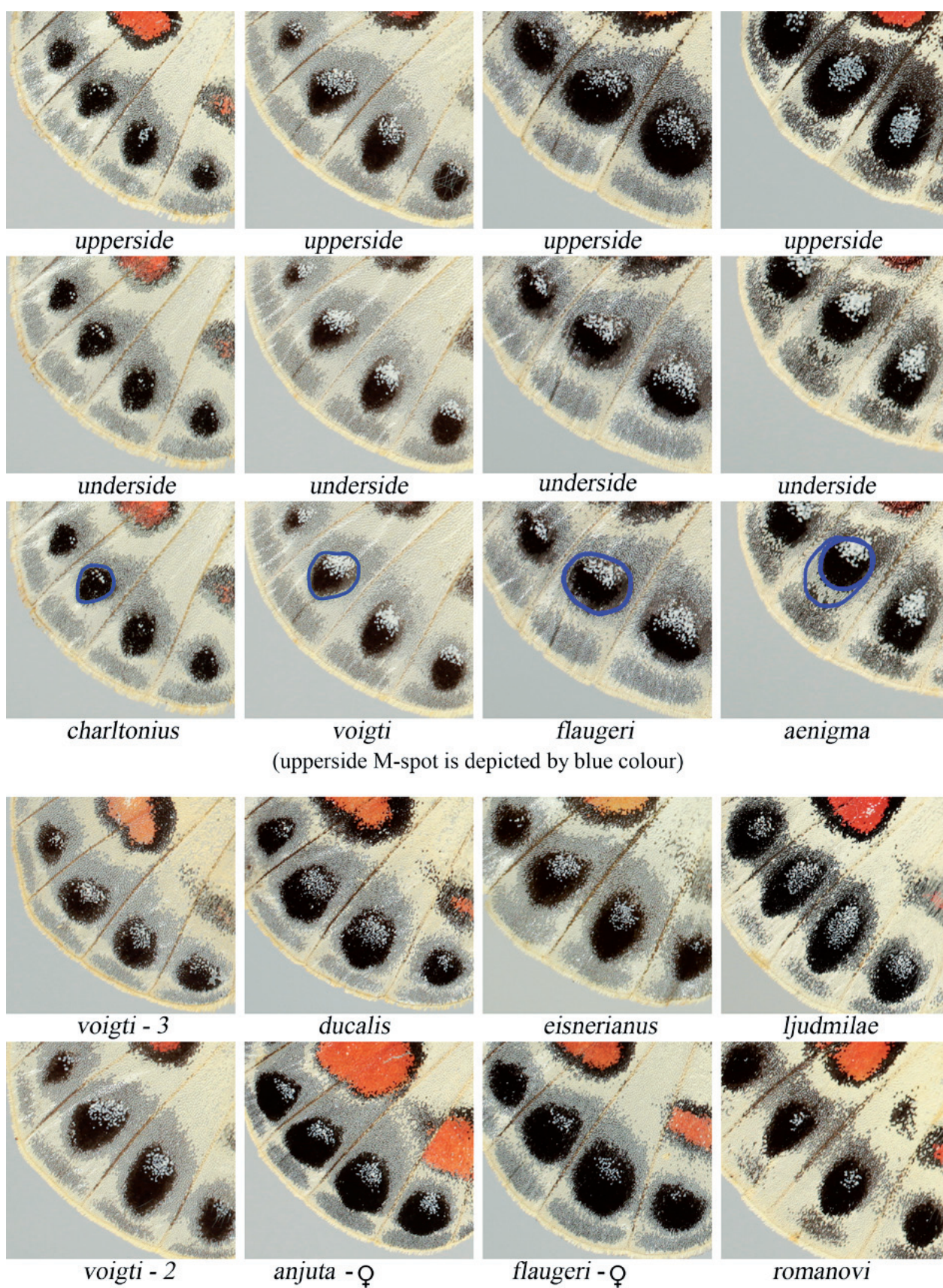


Plate 3. *Parnassius* species, HW: size and form of submarginal spots (additional data - Table III).

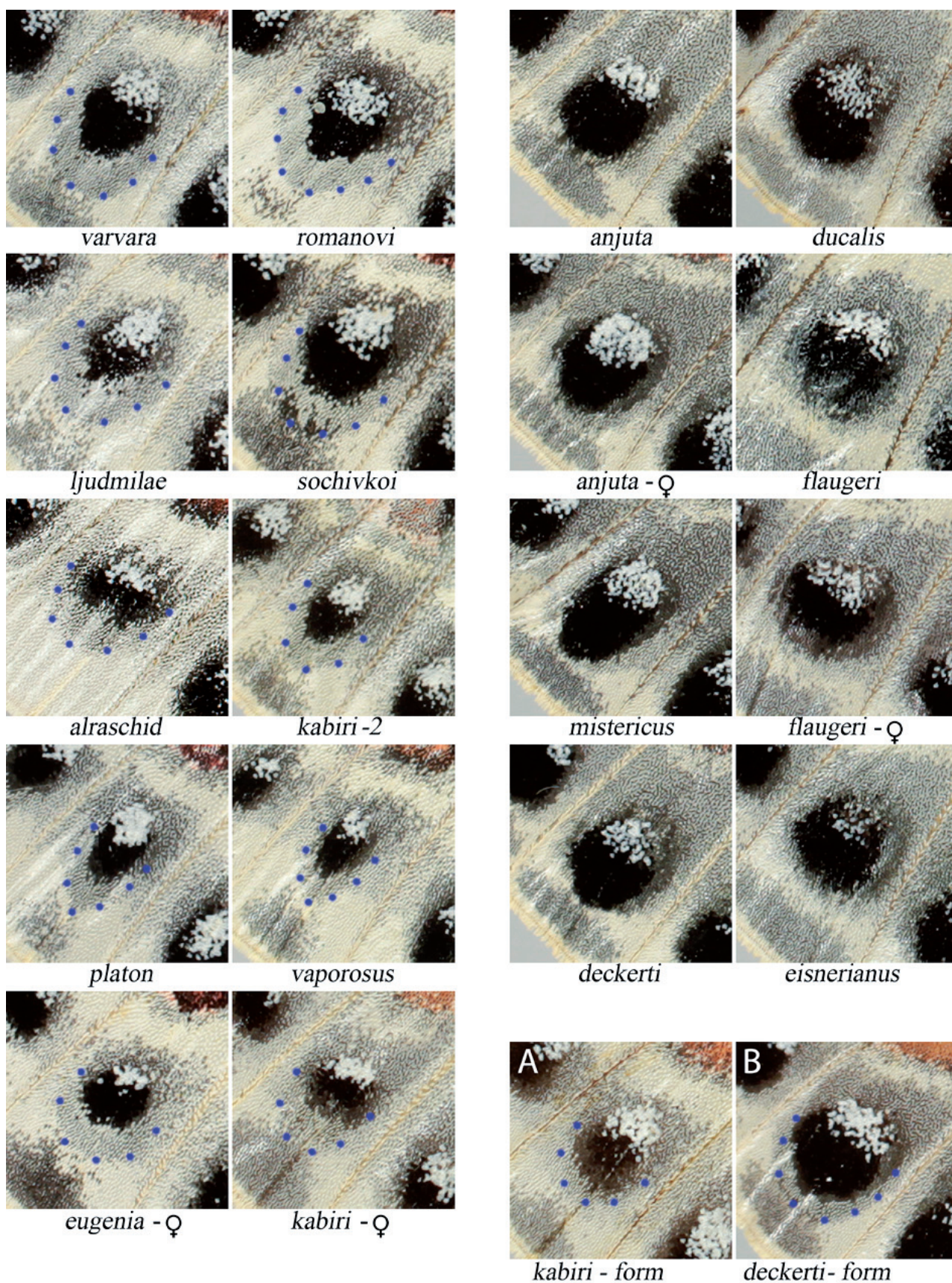


Plate 4. *Parnassius* species, M- submarginal spot, HW underside (upperside M-spot is depicted by blue dots); two photos (A, B) present the forms with abnormal size of the spot (additional data - Table III).

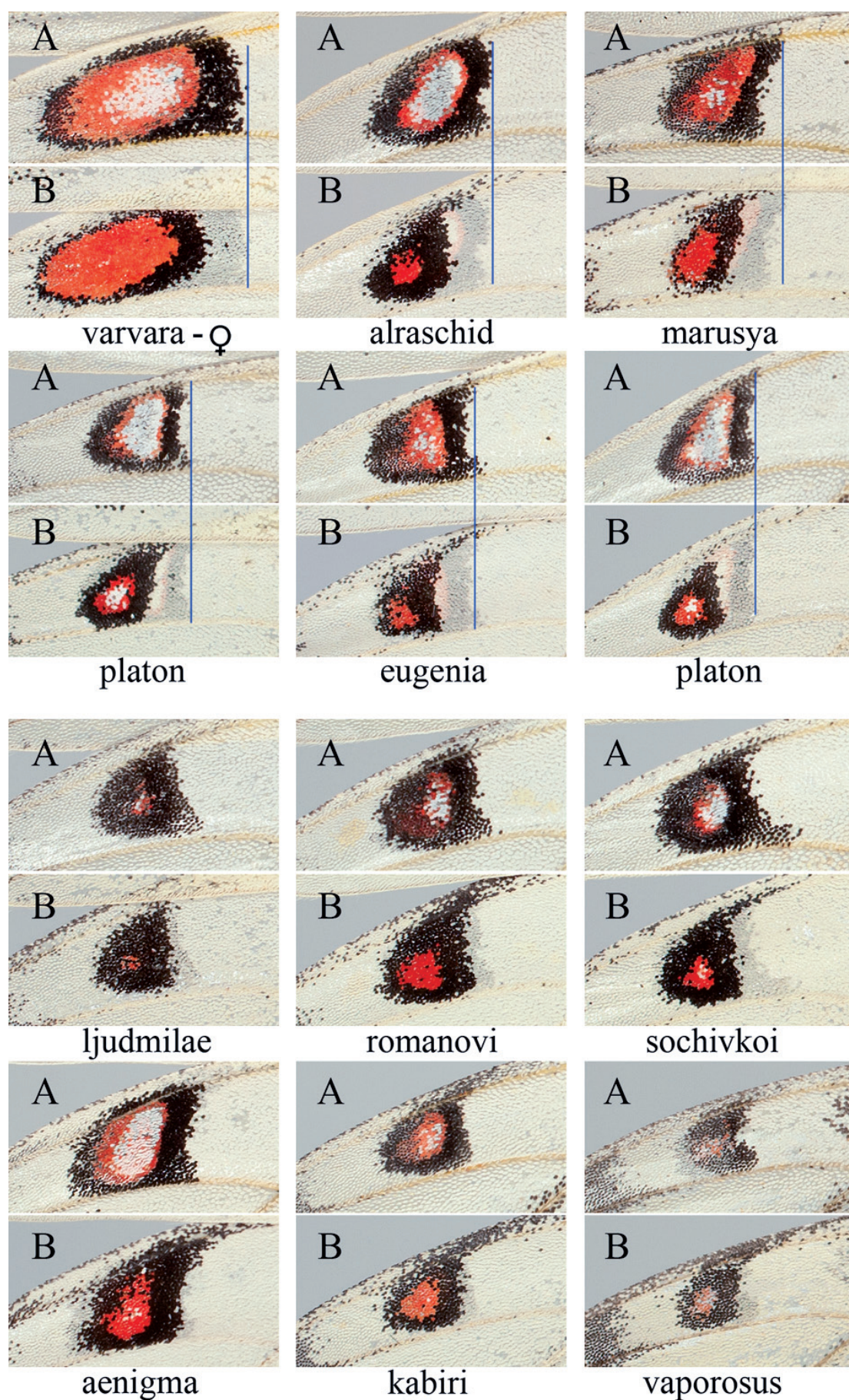


Plate 5. *Parnassius* species, size of the costal red spot at the HW upperside (A) and HW underside (B), different sub-species of the *romanovi* group (additional data - Table III).



Plate 6. *Parnassius* species, representatives of *romanovi* group (left row - ♂♂, right row - ♀♀): 1,2 - *P. r. aenigma* DUBATOLOV & MILKO, 2003 **comb. nov.**; 3,4 - *P. r. varvara* CHURKIN, 2009 **comb. nov.**; 5, 6 - *P. r. alraschid* CHURKIN & PLETNEV, 2012 **comb. nov.**; 7,8 - *P. r. sochivkoi* CHURKIN, 2009 **comb. nov.**; 9, 10 - *P. romanovi romanovi* GRUM-GRSHIMAILO, 1885 **stat. nov.** (additional data - Table III).



Plate 7. *Parnassius* species: 1-5 - *P. romanovi vaporosus* AVINOV, 1912 **comb. nov.** (Tajikistan, Darwas system, Obimazor r., Mazorsky Range, 2011/2013); 6-10 - *P. romanovi marusya* CHURKIN & PLETNEV, 2012 **comb. nov.** (Tadjikistan, Obikhingou r., 2011/2013).

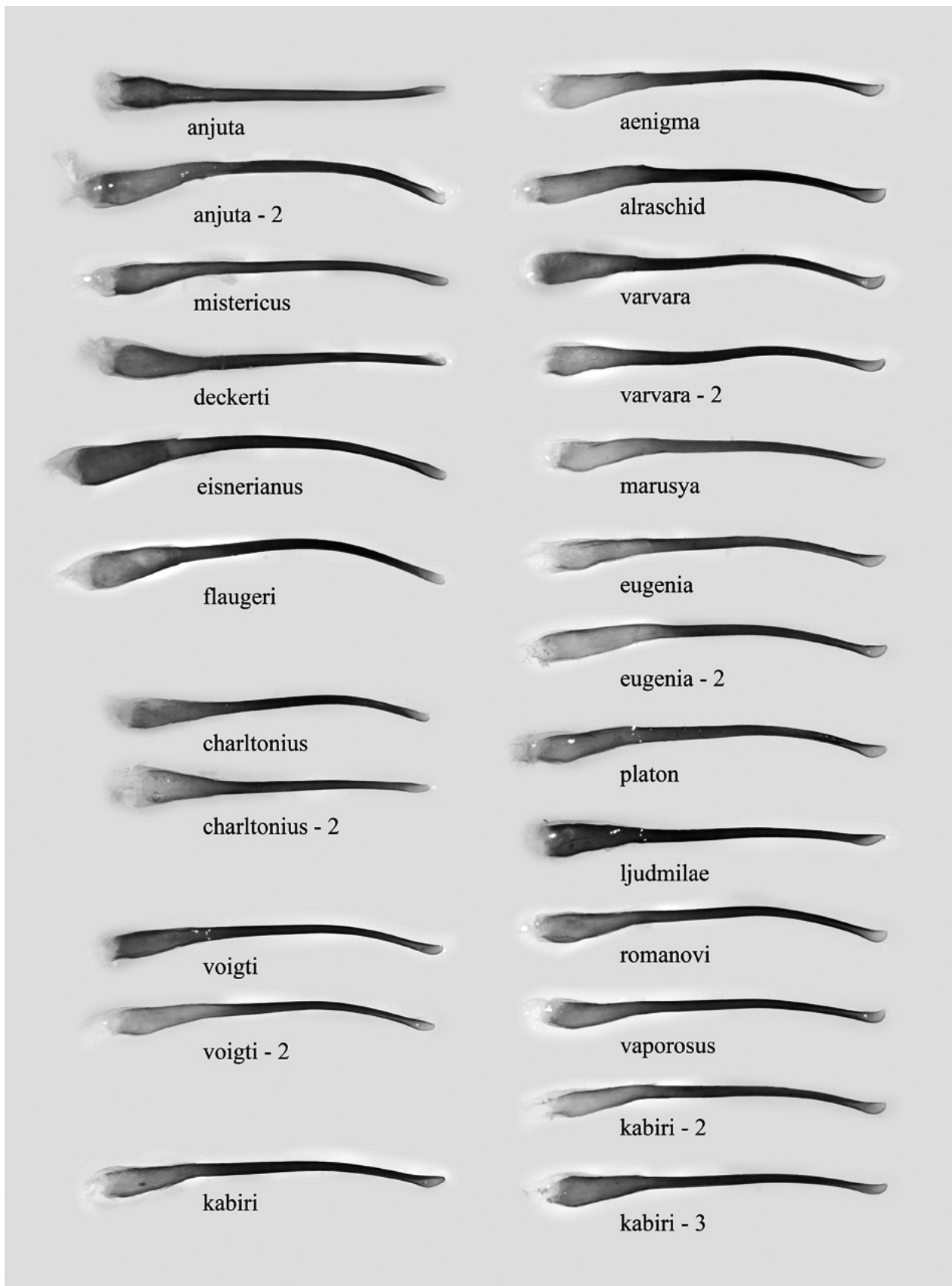


Plate 8. *Parnassius* species, aedeagus (additional data - Table III).

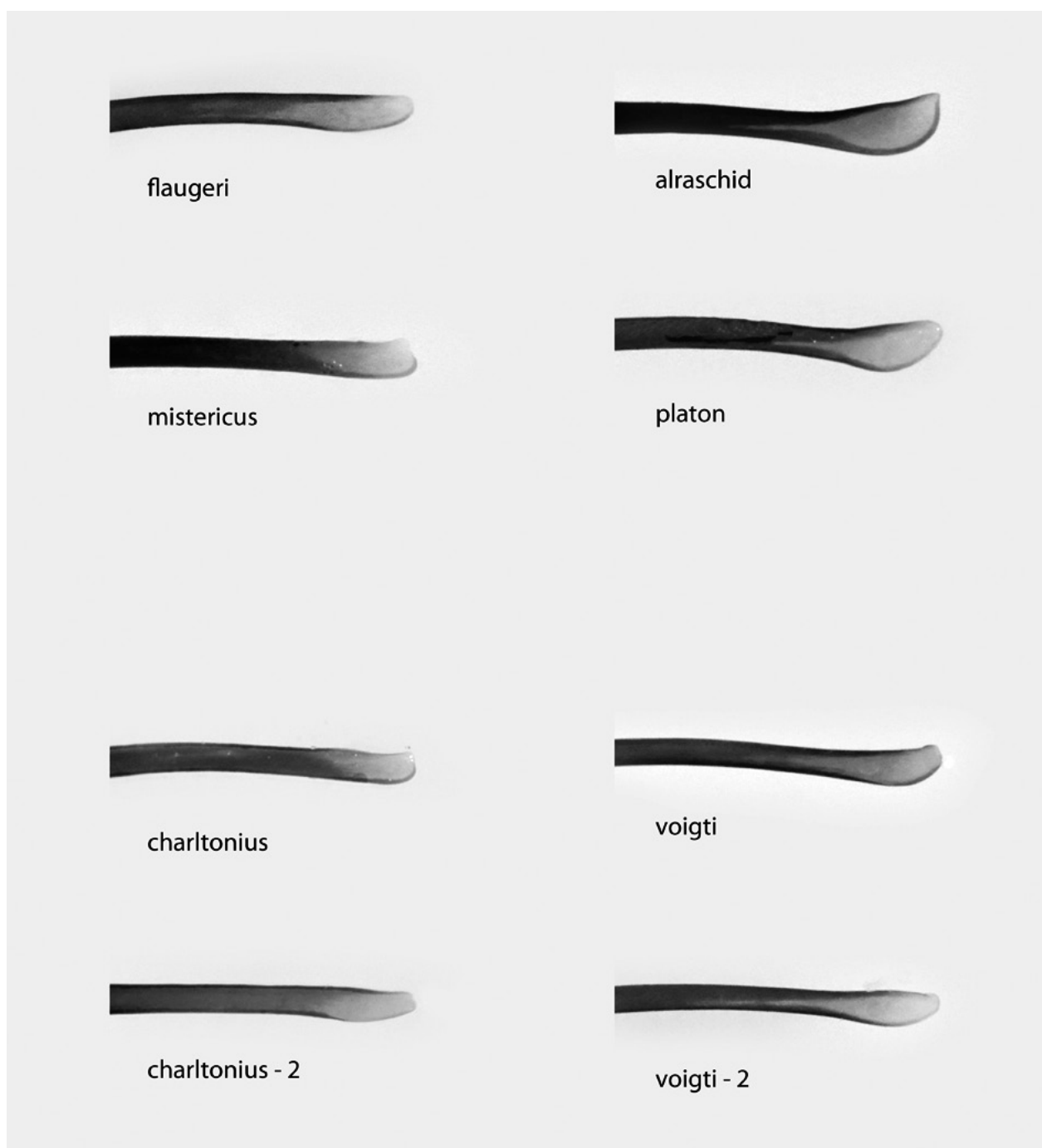


Plate 9. *Parnassius* species, distal part of the aedeagus (additional data - Table III).



Plate 10. *Parnassius* species, ♂ genitalia, dorsal view (additional data - Table III).

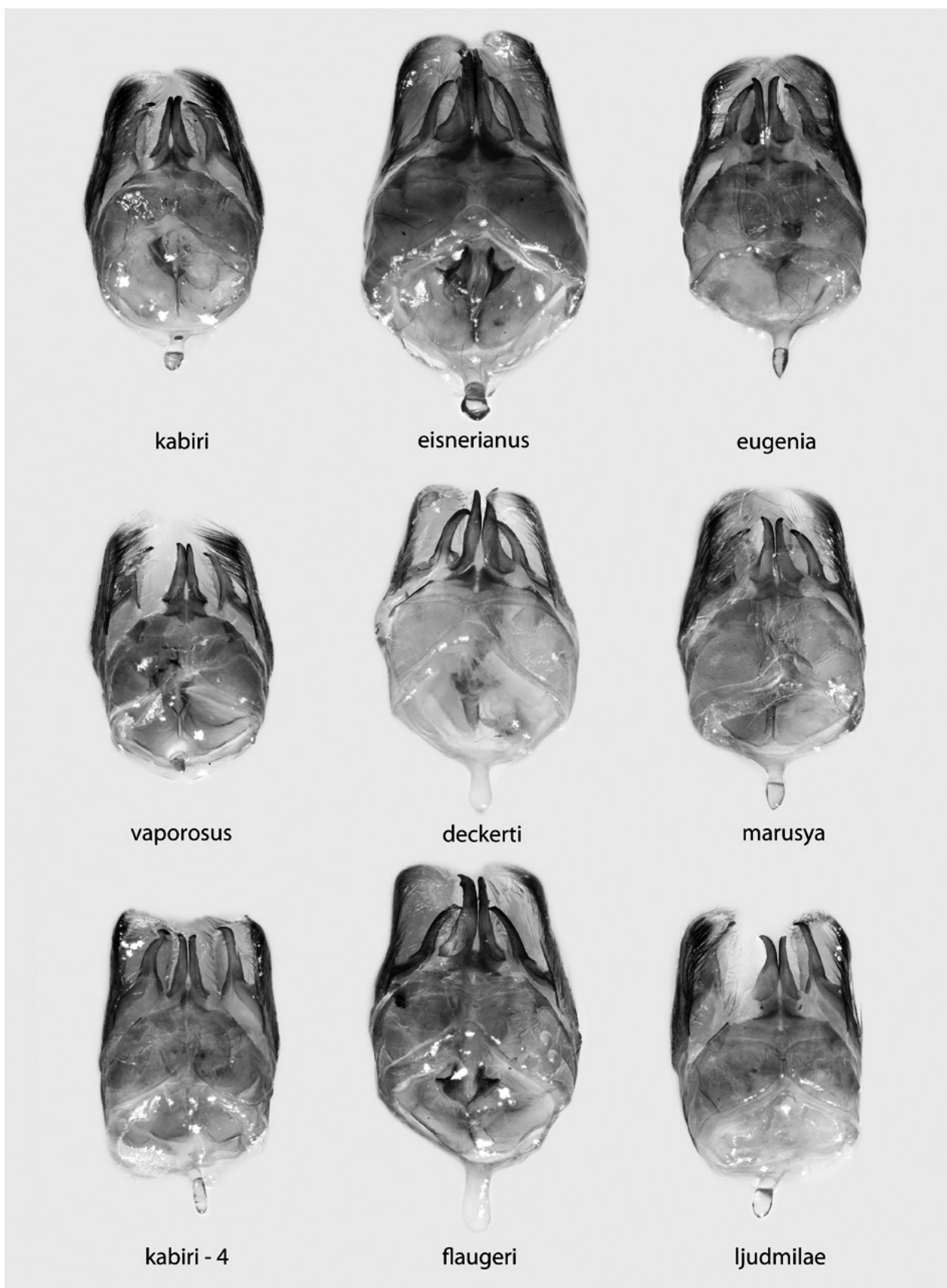


Plate 11. *Parnassius* species, ♂ genitalia, dorsal view (additional data - Table III).

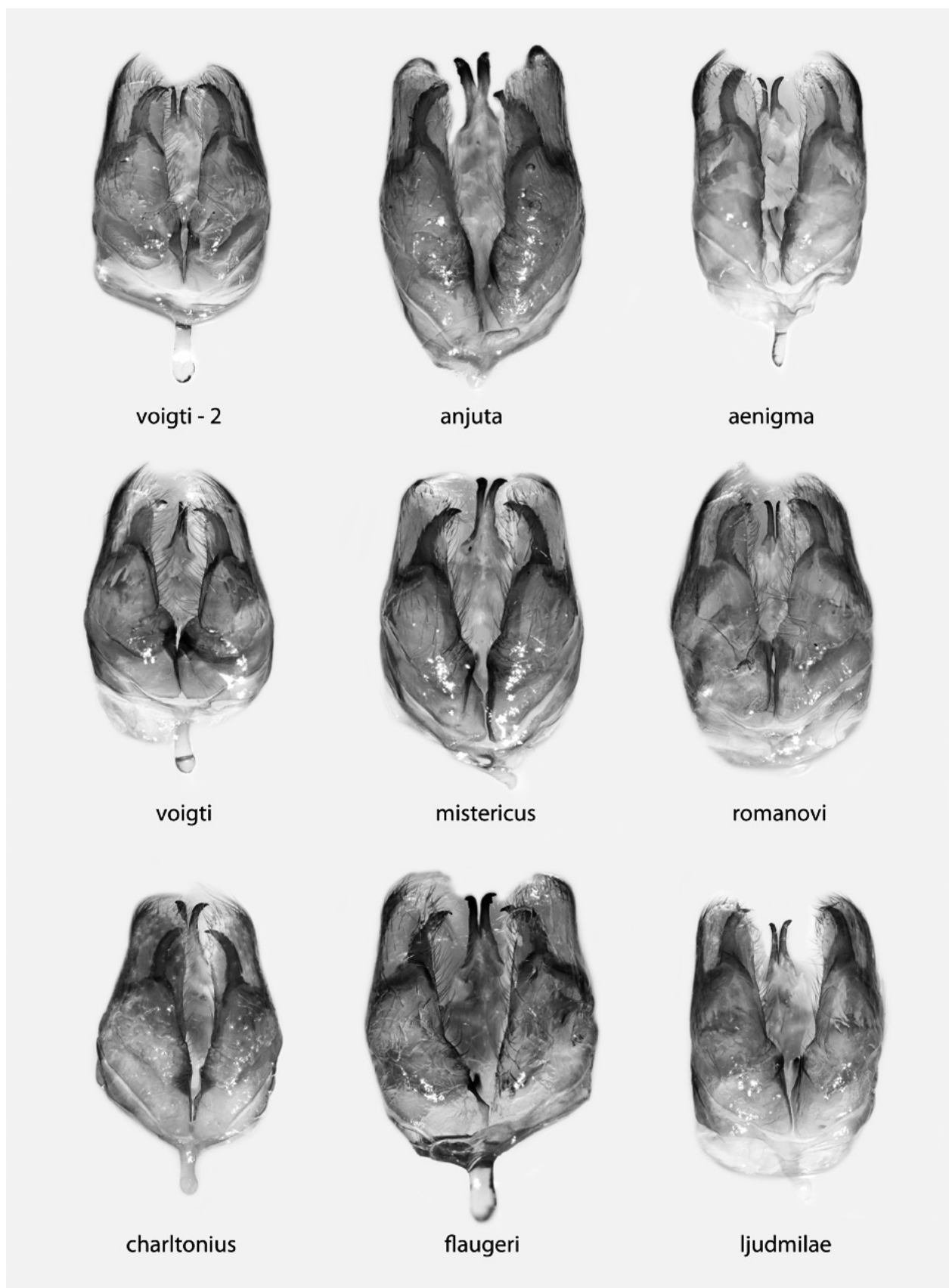


Plate 12. *Parnassius* species, ♂ genitalia, ventral view (additional data - Table III).

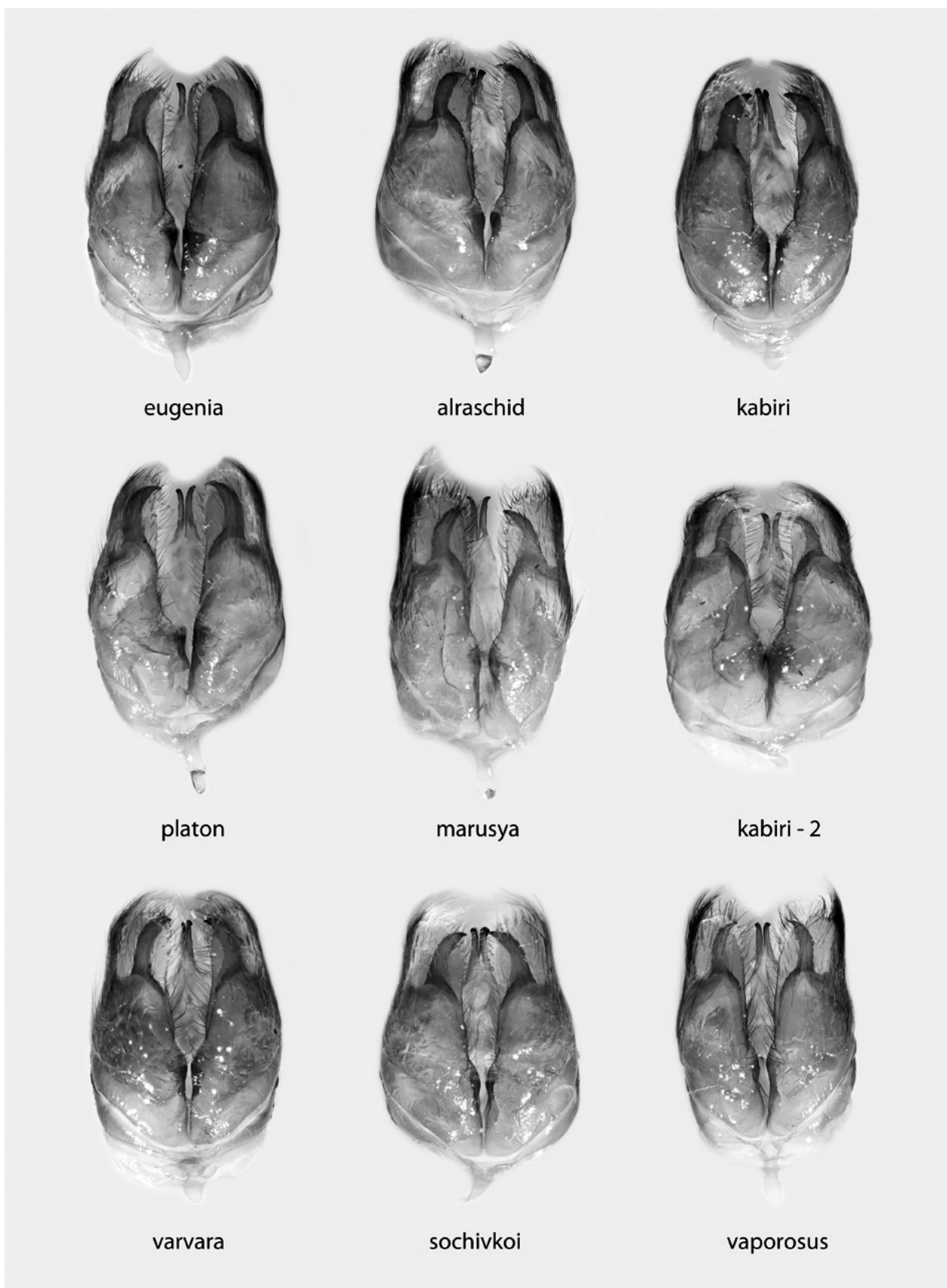


Plate 13. *Parnassius* species, ♂ genitalia, ventral view (additional data - Table III).

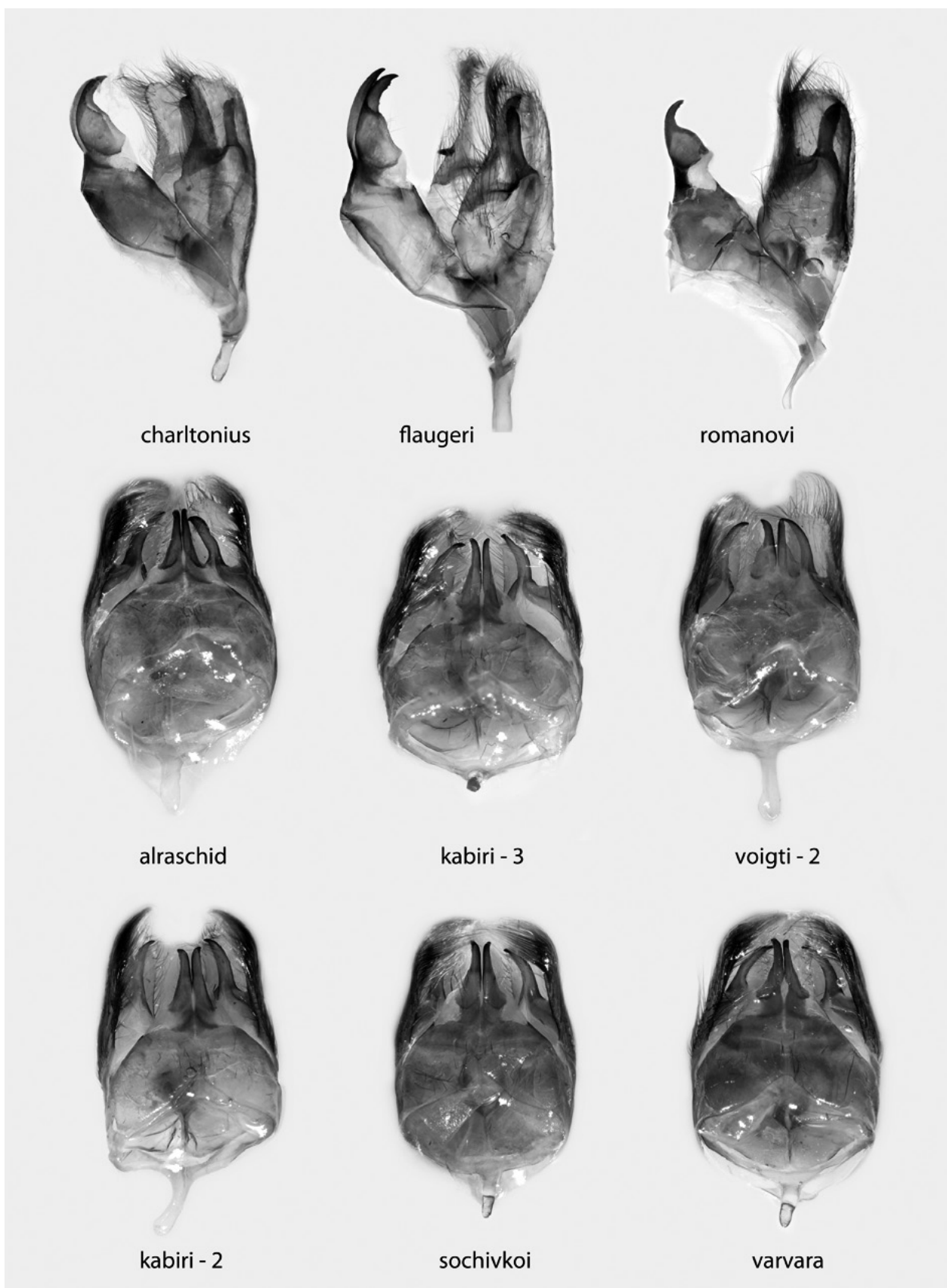
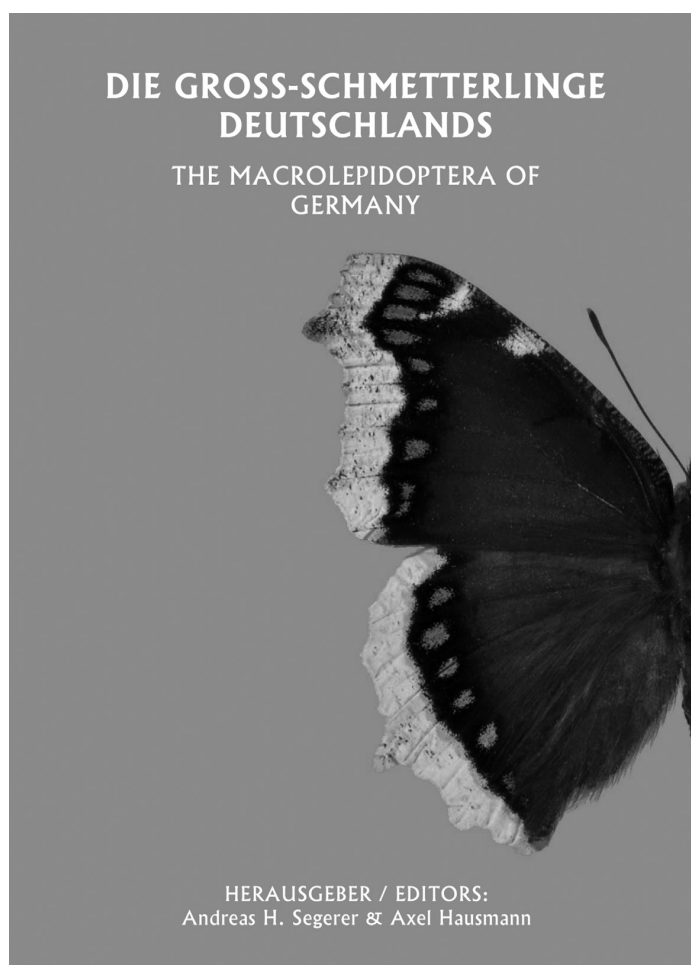


Plate 14. *Parnassius* species, ♂ genitalia: upper row - lateral view, two other rows - dorsal view (additional data - Table III).

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Dieses Buch präsentiert eine vollständige systematische, kommentierte Liste der aus Deutschland bekannten Tagfalter und Großnachtflatter (Insecta: Lepidoptera: Papilionoidea und Macroheterocera) auf neuestem taxonomischem und faunistischem Stand, einschließlich der Einwanderer, Irrgäste und eingeschleppten Arten. Der zweisprachige Text (deutsch/ englisch) wird begleitet von 6171 hochauflösenden Farbfotografien auf insgesamt 183 Tafeln, die die Variationsbreite der insgesamt 1377 behandelten Arten dokumentieren. - This book presents a complete systematic, annotated checklist of the butterflies and larger moths of Germany (Insecta: Lepidoptera: Papilionoidea and Macroheterocera), up-to-date with taxonomy and faunistics, including immigrants, vagrants and artificially introduced species. The bilingual text (German/English) is accompanied by 6171 high-resolution photographs on a total of 183 plates, well reflecting the variability of the 1377 species dealt with.



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